JOURNAL OF AGRICULTURAL RESEARCH

VOL. XV WASHINGTON, D. C., NOVEMBER 18, 1918 No. 7

PHYSIOLOGICAL STUDIES OF NORMAL AND BLIGHTED SPINACH[1]

By RODNEY H. TRUE, OTIS F. BLACK, JAMES W. KELLY, H. H. BUNZELL, LON A. HAWKINS, SAMUEL L. JODIDI, and EDWARD H. KELLOGG, *Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTORY STATEMENT

For some years the growing of spinach (*Spinacia oleracea*) in the trucking sections of Norfolk, Virginia, has been seriously affected by a disease known as "spinach-blight," which is marked by a dwarfing of the affected plants, with a change of color from dark to yellowish green, and the development of a sweet taste and bitterness when the leaf is folded, similar to that seen in a mature tobacco leaf.

This disease has been shown by McClintock and Smith (*29*)[2] to belong to the "mosaic" group. It is therefore a "virus disease" readily communicable from blighted to healthy plants by contact, by injection of plant extracts, and especially by aphids. These insects are responsible for the rapid spread of the disease in the field.

Earlier work by Harter (*16*) and others on the malnutrition of truck crops has led to the belief that this spinach trouble was attributable to the lack of lime and humus, with excessive acidity of the soil, and the work reported in these papers was begun in the hope of throwing light on the abnormal physiological reactions observed. In carrying out these plans, laboratory investigations were made of the ash, carbohydrate, and oxidase contents of both normal and blighted plants, as well as a more fundamental study of the nitrogen metabolism.

Since the nutrition of the plants is closely connected with the condition of the soil in which they grow, and since it has been suggested that the occurrence of the disease might in some degree be influenced by soil conditions (*16*, *17*), it was deemed necessary to take these possible factors into account. The results of an examination of field conditions by Dr.

[1] The investigations here presented bear on different phases of the same problem, although carried out by different workers. Since different men are responsible for the results presented, requiring as they do different types of technic and different lines of special interest, the results are presented in separate chapters in which both the responsibility and the credit of authorship are separately indicated.

[2] Reference is made by number (italic) to "Literature cited," pp. 405-408.

Jay A. Bonsteel, Soil Specialist of the Bureau of Soils, seemed to exclude faulty drainage from the list of possible causes.

The studies reported in this series of papers were carried out in cooperation with the Office of Cotton, Truck, and Forage-Crop Disease Investigations and with the Virginia Truck Experiment Station. From both organizations highly appreciated help was received. The writers are especially indebted to Mr. J. A. McClintock, then Pathologist at the Virginia Truck Experiment Station. He took notes on field conditions, selected and prepared authentic material for laboratory study, and aided in many other ways.

It is believed that this biochemical study of a "mosaic" disease will perhaps have fully as great an interest for plant pathologists as for physiologists.

RODNEY H. TRUE,
Physiologist in Charge of Plant Physiological and Fermentation Investigations.

ASH CONTENT IN NORMAL AND IN BLIGHTED SPINACH

By RODNEY H. TRUE, *Physiologist in Charge*, OTIS F. BLACK, *Chemical Biologist*, and JAMES W. KELLY, *Laboratory Technician, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry.*

In view of the fact that disease conditions not infrequently profoundly modify the demands of the organism for mineral constituents, a study of the ash was undertaken in the hope of getting some light on the nature of the abnormal conditions set up in the spinach by the disease-producing agent.

Typical normal and diseased material was selected by Mr. McClintock from the fields of truck growers living near the Truck Experiment Station. The plants were collected in February, 1915, the roots and stems being carefully dug out by means of a spade. The adhering soil was immediately washed off as well as could be done in the field, and the plants, well wrapped up, were taken to the laboratory, where they were weighed and spread out to dry.

After they had become thoroughly air-dry, the samples were ashed in an electric oven at a temperature of about 700° C. at a cherry-red color. After the quantity of total ash had been ascertained, its constituents were determined according to the methods of the Association of Official Agricultural Chemists.[1]

TABLE I.—*Ash content of healthy and diseased spinach plants*

[Calculated as percentage of total ash]

Constituent.	Jones farm.				Whitehurst farm.			
	Healthy.		Diseased.		Healthy.		Diseased.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	19.39	6.70	18.23	9.74	21.41	7.50	16.68	8.54
Silicon dioxid (SiO_2)	23.89	20.70	35.66	19.76	32.41	18.00	35.99	23.03
Red manganese oxid (Mn_3O_4)	.16	.31	.24	3.38 (?)	.10	.90	.27	1.22
Calcium oxid (CaO)	6.48	4.90	11.88	7.01	4.62	6.07	11.63	9.60
Magnesium oxid (MgO)	3.47	5.35	4.61	9.96	4.43	4.90	4.52	9.19
Potassium oxid (K_2O)	32.06	14.06	23.91	35.19	26.38	12.40	22.60	59.95
Sodium oxid (Na_2O)	10.03	24.64	11.32	5.35	13.70	24.47	10.99	
Sulphur trioxid (SO_3)	3.14	2.70	1.87	2.94	3.00	6.10	1.99	3.55
Phosphorus pentoxid (P_2O_5)	6.71	13.40	6.36	15.15	6.19	14.20	4.05	13.55
Aluminium oxid (Al_2O_3)	2.59	2.69	4.41	2.51	4.03	2.37	3.49	1.66
Ferric oxid (Fe_2O_3)	.51	.93	.56	.96	.67	.92	.50	1.78

The results are shown in Tables I and II. In Table I the total ash is given as percentage of the total weight, air-dry, and the constituents

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted in 1912.

are given in percentages of the total ash. In Table II the total ash, and likewise the constituents, are calculated in percentages of the dry weight of the plant material.

TABLE II.—*Ash constituents of healthy and diseased spinach plants*

[Calculated as percentages of dry weight]

Constituent.	Jones farm.				Whitehurst farm.			
	Healthy.		Diseased.		Healthy.		Diseased.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	19.39	6.70	18.23	9.74	21.41	7.50	16.68	8.54
Silicon dioxid (SiO_2)	4.63	1.39	6.50	1.92	6.94	1.35	6.00	1.96
Red manganese oxid (Mn_3O_4)	.031	.021	.043	.33	.021	.067	.045	.104
Calcium oxid (CaO)	1.26	.33	2.17	.68	.99	.46	1.94	.82
Magnesium oxid (MgO)	.67	.36	.84	.97	.95	.37	.75	.78
Potassium oxid (K_2O)	6.22	.94	4.36	3.43 (?)	5.65	.93	3.80	5.12
Sodium oxid (Na_2O)	2.40	1.65	2.06	.52	2.93	1.84	1.83	
Sulphur trioxid (SO_3)	.61	.18	.34	.29	.64	.46	.33	.30
Phosphorus pentoxid (P_2O_5)	1.30	.90	1.16	1.48	1.33	1.07	.68	1.16
Aluminium oxid (Al_2O_3)	.50	.18	.80	.24	.86	.18	.58	.09
Ferric oxid (Fe_2O_3)	.122	.062	.102	.093	.143	.069	.083	.152

While the quantity of total ash is not strikingly different in normal and in diseased material, the normal tops in all cases seem to be a little richer than the diseased tops, whereas the roots of the diseased plants have somewhat more ash than the normal roots. The great excess of ash in the leaves over the roots is in agreement with the general rule and is seen in both kinds of material.

It is interesting to note that spinach leaves have been found by others to contain an unusually large quantity of total ash, belonging in the same class as tobacco leaves (*Nicotiana tabacum*), hop leaves (*Humulus lupulus*), head lettuce (*Lactuca sativa*), forage-beet tops (*Beta vulgaris*) and *Elodea canadensis* in containing from 16.4 to 20 per cent of total ash. Wolff (*49, p. 141–150*) reports the average for spinach to be 16.48 per cent. The writers find the average of normal samples taken from two fields to be 20.4 per cent, the blighted 17.45 per cent. Thus, the tops of the normal plants are markedly richer in total ash than are those of the diseased plants. This relation is reversed in the roots, the blighted plants averaging 9.14 per cent, in comparison with 7 per cent in the normal material.

Concerning the individual constituents present, a number of points are worth noticing. The case of the silica content is one of especial interest. Although all usual precautions were taken to remove adhering soil, the silica present makes up one of the chief components of the ash, both in leaf and root. As may be seen in Table II, the tops contain several times

as much as the roots in all samples. The proportion of silica present seems to he little affected by health or disease either in tops or roots.

It is interesting to note that in the analyses reported hy Wolff (*49*, *p. 141–150*), the average silica content of this plant, presumably of the tops, is but 4.52 per cent of the total ash against 28.15 per cent found here, and but 0.745 per cent of the total dry weight of the plant against 5.78 per cent found in the normal Norfolk tops. It would he interesting to know in how far the silica content of spinach varies with the locality in which it is produced. A high silica content seems in general to characterize the grasses and grains and not such succulent vegetables as spinach. In this material, however, we have a quantity present equaliog that characteristic of the grains (*49*).

In this connection it is interesting to note Bertrand's (*5*) conclusion that the oxidase activity of plant tissues is related to the manganese content. Since the oxidase relations of normal and blighted spinach are dealt with in a separate paper (p. 377), it is sufficient to state here that in general the higher manganese ash content seems to accompany the stronger oxidase reaction in agreement with Bertrand's observations.

The tendency of calcium to become localized in the leaves in greater quantity than in the roots is seen in hoth normal and blighted samples. While in the normal material the average proportion of the total ash made up hy calcium is about the same in tops and roots, in the diseased plants the greater proportion is clearly found in the tops. When the actual quantity of calcium present in a unit of dry weight is considered, the tops are seen to carry more than three times as much as the roots in both normal and hlighted material and the diseased plants contain nearly twice as much calcium as the corresponding structures of the normal plants. There is here then a tendency of the diseased plants toward increased accumulation of calcium in both tops and roots. It may be noted in passing that according to the analyses given by Wolff (*49*, *p. 141–150*) spinach helongs among those plants which absorb calcium in rather limited quantities, like many of the common legumes. This conclusion is confirmed hy the results here given.

Magnesium is present in both roots and tops of the hlighted plants in nearly double the proportion of the total seen in the corresponding parts of normal plants and in both types of material forms a larger proportion of the ash of the roots than of the tops.

When considered with reference to the actual quantity of magnesium present in a given weight of dry material, that seen in the tops is about the same whether diseased or normal, the quantity seen in normal roots being less than that seen in roots of hlighted plants.

The magnesium content found here is roughly ahout one-half that reported by Wolff (*49*), who lists spinach among the plants which absorb relatively large quantities of this substance.

One of the most striking features of this material, normal as well as blighted, is the high potassium content. While Wolff reports spinach as containing 2.729 per cent of potash, calculated on the dry weight of the material, the average percentage found in the normal tops here is 5.93 per cent and 4.08 per cent in the blighted tops. The roots seem to contain less than the tops both in health and in disease, but there seems to be more in the diseased than in the healthy roots.

Sodium, which is present in less quantity than potassium, seems to be more uniformly distributed throughout the plants. Like potassium, it is usually more abundant in the tops than in the roots in both healthy and diseased samples. Sodium is present in less quantity than potassium in the tops, but in some cases this relation is reversed in the roots. Wolff (*49*) reports 5.816 per cent of sodium, a quantity, presumably in the tops, about double that seen here in the normal tops. In any case, spinach seems to absorb an unusual quantity of sodium, betraying clearly its halophytic tendencies.

The sulphate radical here, as in most plants, is rarely absorbed in large quantity. According to Wolff (*49*), it reaches 1.113 per cent of the dry weight of the plant material. Here the quantity found in the normal tops is less than half of that amount, which in turn is about double that found in the blighted tops. The roots are poorer than the corresponding tops in each individual case.

Phosphate absorption is not heavy in spinach at the stage in which the disease appears, but seems to be influenced by the blight. In the tops the average normal phosphate content is 1.31 per cent of the dry weight of the plant, against 0.92 per cent in the blighted tops. This relation is completely reversed in the roots, the normal samples containing 0.98 per cent, against 1.32 per cent in the blighted roots.

These results, which agree fairly well with Wolff's data, place spinach with head lettuce and cauliflower hearts (*Brassica oleracea botrytis*) near the top of the list of leafy vegetables in the quantity of phosphates absorbed.

Aluminium, rarely absorbed in great quantity, is present in spinach in small amounts. The normal and blighted tops contain alike nearly 0.7 per cent, calculated on dry weight, while the roots in samples of both kinds agree in containing about 0.18 per cent each. According to Berthelot and Andre (*4*), the roots of plants usually contain more aluminium than the leaves.

It is probable that the material here studied is unusually high in aluminium, since Czapek (*12, p. 855*) reports that, as a rule, a content of more than 0.5 per cent of the total pure ash is not found. Here the aluminium makes up about 3.3 per cent of the total ash in the normal tops and nearly 4 per cent in the blighted tops. The proportion of aluminium to total ash is less in the roots than in the tops in health and in disease, one case excepted.

Wolff (*49*) reports spinach to have an average iron content of 0.552 per cent, calculated on the dry weight of the plant material, and with even this small quantity it is much richer in this element than the vast majority of plants that had then been studied.

It is interesting to note that healthy spinach tops were here found to contain an average of 0.132 per cent of iron, calculated on the dry weight, against 0.095 per cent in the blighted tops, in either case a quantity much larger than that recorded by Wolff. The roots of the diseased plants were found to contain an average of 0.122 per cent of iron and the roots of the normal plants 0.065 per cent. It seems as though a part of the iron that entered the plant through the roots accumulated there instead of going up to the leaves, as in the normal plants. It is interesting to note also that when calculated as percentage of the total ash the iron content of the roots always exceeds that of the corresponding tops, a relation more in evidence in diseased than in healthy roots.

OXIDASE REACTION IN HEALTHY AND IN BLIGHTED SPINACH

By H. H. BUNZELL, *Formerly Chemical Biologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry*

A few years ago it was observed by Hasselbring and Alsberg (*18*) that spinach grown in the market gardens near Norfolk, Va., and affected by a disease resembling the mosaic of tobacco, had a greater oxidase content than healthy spinach from the same region. This observation coincided with the work by Woods (*50, 51*) on the mosaic disease of tobacco. Since that time the writer has developed a quantitative method for the determination of oxidases, utilizing atmospheric oxygen (*7*). It was decided therefore to extend the observations of Hasselbring and Alsberg by comparing the oxidase activity of the leaves and roots of the diseased plants with those of healthy plants.

Three different collections of samples were made, designated as Set I, II, and III, respectively. In each case typically diseased plants were selected, as well as healthy control plants grown in the immediate vicinity of the diseased spinach. In all instances, therefore, the healthy and the diseased samples of the same set were grown under the same climatic and soil conditions. The plants were carefully washed in the laboratory to remove any adhering soil, and the surface water was removed by blotting the plants between sheets of filter paper. The leaves, freed from petioles and midribs, were dried over lime in a vacuum at room temperature. The roots were cut into pieces 2 to 3 mm. long and dried in a similar way. The samples were dried to constant weight and then powdered until the whole of the sample passed through a sieve of 100 meshes to the inch, after which they were kept in a desiccator.

The experiments were carried out according to the method formerly described (*10*). The following reagents were used: Pyrogallol, pyrocatechol, hydroquinone, phloroglucin, guaiacol, tyrosin, meta-cresol, para-cresol, eugenol, and isoeugenol. The temperature at which the experiments were carried out was 37.4° C., and the rate of shaking five complete excursions in 3.4 seconds.

In most of the experiments the quantity of dry material used was so chosen as to give a reading of about 2 cm. In none of the experiments, however, was more than 0.10 gm. of the powder used, so that the readings were considerably below 2 cm. in the slightly active or inactive preparations. To make the results comparable, they were all calculated on the basis of 0.10 gm. of powder. The reagents were used in quantities ranging from 0.01 to 0.02 gm., this being an excess of the reagent in all cases. The results obtained are given in Table I.

TABLE I.—*Relative oxygen absorption of various oxidase reagents in the presence of healthy and diseased spinach material*

Material.	Pyrogallol.	Pyrocatechol.	Hydroquinone.	Phloroglucin.	Guaiacol.	Tyrosin.	Metacresol.	Paracresol.	Eugenol.	Isoeugenol.
SET I.										
Leaves:										
Normal.....	0.70	3.20	0.20	0.02	0.00	0.00	0.05	0.85	0.00	0.00
Pathological..	1.20	4.55	.52	.25	.10	.25	.27	.88	.00	.00
Roots:										
Normal.....	2.32	4.00	9.00	3.50	4.23	6.70	2.00	18.20	.00	.00
Pathological..	2.60	5.20	8.20	3.03	4.25	8.00	5.23	28.60	.00	.00
SET II.										
Leaves:										
Normal.....	1.40	5.40	.20	.00	.00	.00	.00	1.75	.00	.00
Pathological..	1.50	5.55	.55	.50	.32	1.00	.92	5.27	.02	.20
Roots:										
Normal.....	2.25	4.68	8.13	3.20	2.43	6.40	3.13	26.50	.07	.00
Pathological..	2.60	6.00	9.87	3.10	3.50	8.40	5.20	31.50	.10	.17
SET III.										
Leaves:										
Normal.....	.90	1.80	.33	.00	.00	.00	.00	.72	.00	.00
Pathological..	1.27	5.90	.23	.00	.00	.05	.07	1.52	.00	.00
Roots:										
Normal.....	1.15	1.90	4.00	2.20	1.50	4.60	2.63	20.70	.00	.00
Pathological..	2.27	2.80	7.00	3.60	2.20	8.20	2.40	31.50	.00	.10

Inasmuch as the various samples had different moisture contents, the results given in Table I are not strictly comparable. They were made so, however, by means of a calculation based on the following reasoning: If it be assumed that the total oxidase activity of the plant material is in the juice, and the weight of the solids dissolved in the juice be neglected, the oxidizing power of 0.10 gm. of juice can be calculated from the equation

$$a\ (\text{juice}) = a\ (\text{solids})\ \frac{\text{percentage of solids}}{\text{percentage of juice}}$$

The oxidizing power of 1 liter of juice is then necessarily 10,000 times the figures obtained in this way. But our unit of activity is the juice, 1 liter of which will transfer 8 gm. of oxygen (*8*), corresponding at 37.4° C. and 76 cm. pressure to 6,367 cc. The volume of the gas in the apparatus was 19 cc. The equation for calculating A, or the activity of the juice present in the fresh leaves and roots, is therefore

$$A = \frac{10{,}000}{25{,}475}\ a\ (\text{solids})\ \frac{\text{Percentage of solids}}{100 - \text{Percentage of solids}}$$

$$A = 0.393\ a\ (\text{solids})\ \frac{\text{Percentage of solids}}{100 - \text{Percentage of solids}}$$

The data which were presented in Table I are given in Table II, recalculated on the basis just described.

TABLE II.—*Oxidase activities of healthy and diseased spinach material*

[Results expressed in units]

Material.	Pyrogallol.	Pyrocatechol.	Hydroquinone.	Phloroglucin.	Guaiacol.	Tyrosin.	M-cresol.	P-cresol.	Eugenol.	Isoeugenol.
SET II.										
Leaves:										
Normal.....	0.087	0.337	0.019	0.000	0.000	0.000	0.000	0.109	0.000	0.000
Pathological..	.102	.379	.038	.034	.022	.069	.063	.359	.001	.014
Roots:										
Normal.....	.178	.371	.645	.254	.193	.508	.248	2.101	.006	.000
Pathological..	.278	.642	1.056	.332	.374	.899	.556	3.370	.011	.018
SET III.										
Leaves:										
Normal.....	.073	.147	.027	.000	.000	.000	.000	.059	.000	.000
Pathological..	.139	.598	.023	.000	.000	.005	.007	.154	.000	.000
Roots:										
Normal.....	.154	.254	.535	.294	.201	.615	.352	2.768	.000	.000
Pathological..	.280	.346	.864	.444	.271	1.024	.420	3.887	.000	.012

Table I shows three instances and Table II only two in which the activity of the healthy leaves or roots was greater than that of the diseased material. In all other cases there was either no measurable activity in both types of material or the diseased material was more active than the corresponding healthy material. This difference was from 50 to 100 per cent.

The figures expressing the activity of phloroglucinase, guaiacolase, tyrosinase, and meta-cresolase, are particularly interesting. These figures seem to indicate qualitative differences.

These results resemble those obtained in several other plant diseases. In the case of the mosaic of tobacco (*51*), the leafcurl of potatoes (*13*), the curly-top of sugar beets (*8*), and the curly-dwarf of potatoes (*9*), the diseased material shows a greater power to transfer atmospheric oxygen to certain aromatic compounds than the healthy material. In all these diseases the most characteristic symptom is a marked stunting of the plant. The following generalization seems therefore justified: In the above-mentioned plant diseases, which cause dwarfing of the plants affected, the capacity of the plant juice to utilize atmospheric oxygen for the oxidation of certain chromogens is abnormally increased. How this increase in the catalytic activity of the cell sap is brought about remains a problem. Whether the peroxid-forming substances are increased so that there is an increase in the oxygenases, which, in the presence of an excess of peroxidases might lead to the results outlined (*3*), or whether a greater quantity of specific activators are formed, which,

combined with various metabolic products form very unstable and readily oxidizable compounds, we are of course as yet unable to judge (*30*). It is possible that the difference observed was primarily of physical origin. Traube (*48*), in his paper on catalysis, recognized surface tension as one of the most important factors in the acceleration of biochemical reactions. The oxidase activity observed might be due simply to an increased concentration of the oxidizable material or oxygen, or both, in the layers adjacent to certain colloidal particles.

It is certain that in the course of stunting of growth there is an increase in the effectiveness of the oxidase mechanism. Whether this is the cause or a symptom of the disease is an open question. If we assume with Palladin (*37*) that with the aid of the respiratory pigments the oxidases are capable of carrying on the process of cell respiration, then it can readily be seen how an increased oxidation of some of the intermediate products of metabolism might seriously alter the course of the latter. Such plants could be said to be in a state of "fever" (*8*). If, on the other hand, oxidases are merely protective agents, as Portier (*38*) assumes, then the increased oxidase activity of the diseased plants would have to be ascribed to an attempt on the part of the plant to rid itself of poisonous products formed in the course of its abnormal metabolism.

CARBOHYDRATE PRODUCTION IN HEALTHY AND IN BLIGHTED SPINACH

By RODNEY H. TRUE, *Physiologist in Charge*, and LON A. HAWKINS, *Physiologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry*

Spinach plants affected with the blight show many symptoms pointing to a derangement of functions concerned with the carbohydrates, among which are the yellowish-green color and the sweetish taste. It was therefore deemed important to investigate these constituents in normal and in diseased plants. The material was taken from commercial fields near the Virginia Truck Experiment Station. The plants were carefully dug with a spade, and the soil adhering to the roots was quickly washed off. To reduce the translocation of products, the plants were divided into roots and tops, and after being loosely packed in market baskets were covered with paper and stored in a building in diffused light. The collection took place late in the forenoon on February 5, 1915, a clear day.

The samples were kept in a cool place while in transit and were taken to the laboratory at 7.30 the next morning, where they were given immediate attention.

Starch, sucrose, and reducing sugars were determined by the usual methods. The results of these determinations are presented in Table I as percentages calculated on both fresh and dry weights of tops and on the fresh weights of the roots.

It will be noted that the samples of diseased tops have a somewhat greater percentage of dry matter than the healthy tops.

The reducing sugars under the conditions here given are clearly less abundant in the tops of blighted plants than in the normal samples, while in the roots but a trace is present in either type of material. The situation with reference to the sucrose in the tops, however, is quite the reverse, the diseased plants containing a considerably greater quantity than those in health. This difference is so great as to give a much higher total sugar content for the pathological material, a fact which in part accounts for the strikingly sweet taste found in the latter plants. It is of interest, however, to note that no noticeable sweetness is found in the normal leaves, although in the material collected in 1915 they contain nearly 80 per cent as much total sugars and nearly 65 per cent as much sucrose. Since the taste of sweetness is interfered with by a variety of other taste sensations, it is possible that certain substances having a marked taste may be present in the normal material and absent in that affected by the disease. On tasting the fresh material in the field it seemed that the characteristic "spinach" taste so strongly marked in the healthy leaves was almost lacking in the sweet diseased leaves.

TABLE I.—*Carbohydrates in healthy and in blighted spinach*

Carbohydrate.	Spinach tops.								Spinach roots, 1915.	
	Wet weight.				Dry weight.				Wet weight.	
	Normal		Diseased.		Normal.		Diseased.			
	1915	1916	1915	1916	1915	1916	1915	1916	Normal.	Diseased.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Dry weights of material	.....	12.53	.....	17.43	.....	.....	.....	.....	.....	.....
	.....	12.87	.....	16.96	.....	.....	.....	.....	.....	.....
	11.90	13.42	11.90	16.10	.....	.....	.....	.....	.....	.....
	11.50	13.95	12.50	18.56	.....	.....	.....	.....	.....	.....
Average	11.70	13.10	12.20	17.26	.....	.....	.....	.....	.....	.....
Reducing sugars	.49	.28	.52	.26	4.10	2.23	4.26	1.49	.....	.....
	.70	.26	.47	.29	5.98	2.01	3.85	1.71	Trace.	Trace.
	.69	.25	.45	.26	5.89	1.86	3.68	1.61	.....	.....
	.40	.22	.44	.24	4.10	1.58	3.60	1.29	.....	.....
Average	.50	.25	.47	.26	5.02	1.89	3.85	1.50	.....	.....
Total sugars	1.46	2.48	2.08	4.77	12.47	19.79	17.04	27.36	.....	.....
	1.36	1.88	2.18	5.22	11.62	14.61	17.86	30.37	4.07	6.42
	1.16	1.60	1.89	5.49	9.91	11.92	15.49	34.10	5.94	4.17
	2.03	2.33	1.83	4.74	17.36	16.70	15.00	25.53	.....	.....
Average	1.50	2.07	1.99	5.05	12.84	15.69	16.35	29.26	5.00	5.30
Sucrose calculated by difference	.93	2.20	1.50	4.51	7.94	17.56	12.29	25.87	.....	.....
	.63	1.62	1.64	4.93	5.44	12.60	13.44	29.07	.....	.....
	.45	1.35	1.38	5.23	3.84	10.06	11.31	32.49	.....	.....
	1.47	2.11	1.33	4.50	12.56	15.12	10.90	24.24	.....	.....
Average	.88	1.82	1.46	4.79	7.44	13.80	11.98	27.76	.....	.....
Starch	.95	1.06	1.53	1.72	8.11	8.45	12.54	9.87	.....	.....
	.79	1.13	1.51	1.93	6.75	8.78	12.37	11.38	2.03	2.26
	.73	.99	1.48	1.57	6.24	7.38	12.13	9.75	2.51	2.58
	.88	.99	1.51	1.98	7.52	7.10	12.37	10.67	.....	.....
Average	.84	1.04	1.51	1.80	7.15	7.88	12.35	10.43	2.27	2.42

The starch content of the diseased tops is somewhat more than double that of the normal material. In the roots, both total sugars and starch were practically alike in both types of material.

From these results it appears justifiable to conclude that the cause of injury does not affect the machinery of photosynthesis or the materials used in carbohydrate manufacture to such an extent as to stop production. That this is carried on with equal efficiency in all parts, or with normal efficiency even in any part of the leaf, however, can not be stated. Indeed, the yellowish-green color representing an apparent reduction of chlorophyll would seem likely to go with a decreased activity in the

photosynthetic function. This condition recalls that of tobacco leaves when "mature" for cutting. The color changes to a more yellowish green, the leaves take on the brittle character seen in the diseased spinach and like it become gorged with starch.

It would hardly be safe to assume that photosynthetic activity is not impaired in the blighted plants in spite of the accumulation of carbohydrates. It is quite possible that impairment may be the case and that accumulation results from some interference with carbohydrate utilization.

In view of the destructive action of oxidases on diastatic enzyms, reported by Woods (51) in the case of tobacco mosaic, it was thought possible that here a somewhat similar situation was present. Since Bunzell in his investigation on this subject found the oxidase reaction with most reagents to be somewhat more intense in the diseased material, both leaves and roots, than in normal samples, it was thought necessary to determine the comparative diastatic activity of juices from these two types of material. The fresh leaves, after being ground in a mortar, were placed in a flask having a volume of 250 cc. and digested for 24 hours with 100 cc. of glycerin in an ice box at a temperature of about 10° C. This was then made up to volume, strained, and 50 cc. of the solution was added to 25 cc. of 1 per cent soluble starch. Controls were made in the same way from each sample to which no starch paste was added. One cc. of toluol was added to each flask to prevent the action of microorganisms. All preparations were allowed to stand at 30° C. for 48 hours, after which they were removed, cleared with lead acetate, made up to 100 cc. and filtered. The data given in Table II show the quantity of reducing sugar present in the preparations containing starch paste in excess of the controls from the same samples.

TABLE II.—*Diastatic activity in normal and in blighted spinach*

Date of collection.	Quantity used.	Glucose.	
		Normal leaves.	Blighted leaves.
	Gm.	*Gm.*	*Gm.*
February, 1915	100	0.316	0.308
Do	100	.304	.312
Do	100	.340	[a] .528
Average		.320	.310
March, 1916	50	.0130	.0142
Do	50	.0107	.0155
Do	50	.0172	.0121
Average		.0117	.0106

[a] Extracted with larger volume and calculated to the same basis as the others.

These results seem to point to the absence of any marked difference in the starch-digesting capabilities of normal and blighted spinach. This being the case it would seem to be indicated that the cause of carbohydrate accumulation should be sought in the deeper-lying metabolic processes in connection with which carbohydrates are utilized.

To recapitulate, it appears that in spinach-blight the process of carbohydrate manufacture is not inhibited, although it may be retarded. The reducing sugars are practically absent from the roots of all plants, while in the tops the normal plants contain somewhat more than the diseased. Both sucrose and starch are present in the leaves of the blighted plants in markedly greater quantity than in those of the normal plants. They are found in the roots of both healthy and diseased plants in approximately like quantities.

Determinations of diastatic activity failed to bring out any marked difference between healthy and diseased plants.

It is indicated that carbohydrate accumulation is due not to a breakdown of digestion but to some partial failure in the subsequent metabolic processes in connection with which carbohydrates are used.

NITROGEN METABOLISM IN NORMAL AND IN BLIGHTED SPINACH

By SAMUEL L. JODIDI, *Organic Chemist*, EDWARD H. KELLOGG, *Scientific Assistant*, and RODNEY H. TRUE, *Physiologist in Charge, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry.*

INTRODUCTION

In view of the evident derangement of the functions of nutrition in spinach-blight, it seemed necessary to undertake an investigation of the more fundamental processes of synthetic metabolism in the hope of learning in what way the disease causes injury. The purpose of this paper is to present the results gained from a study of the nitrogen metabolism.

Owing to their great importance in plant metabolism, certain groups of nitrogen-containing compounds demanded attention. The total nitrogen, the polypeptids and the proteins, closely connected as they seem to be with the fundamental activities of life in both plants and animals, the nature and quantity of the nonproteins present, especially of the amino acids, were studied in both healthy and diseased material.

For a number of years it has not been unusual in plant and soil investigations to estimate the protein content of biological materials by determining their nitrogen content (usually by the Kjeldahl method) and multiplying the nitrogen found by the factor 6.25. The literature contains a great number of such protein estimations. Evidently the investigators maintained that the nitrogen present in plant and soil materials is made up solely or chiefly of proteins or protein-like bodies, whose nitrogen percentage does not materially differ from that of proteins. Schulze and his coworkers (*41–45*) were among the first to demonstrate that such is not the case. They have shown, for instance, that a considerable portion of the nitrogen contained in Irish potatoes (*Solanum tuberosum*) and sugar beets, sometimes more than one-half, is made up of nonproteins (acid amids, amino acids, etc.). So far as the writers are aware, no similar systematic investigation concerning the character of the nitrogenous compounds in spinach has been made. Hence, it seemed of considerable importance to find out the nitrogen distribution in the spinach under normal physiological conditions. Furthermore, it was of interest to learn what changes, if any, had taken place in the nitrogen compounds of the spinach under pathological conditions.

Of special interest is the nature of the nonproteins (acid amids, amino acids, polypeptids) occurring in spinach. These compounds may be considered either as products of regressive metabolism in plants, or as products of synthesis in the latter built up out of inorganic nitrogen plant food, and are significant because of their food value, and the func-

83816°—18——2

tions which they perform. Not only do they represent protein-saving materials, since in their presence the animal organism needs less protein for the maintenance of nitrogen equilibrium, but, a fact of still greater interest, recent investigations have demonstrated that animals can be maintained in nitrogen equilibrium, or even gain weight, when they are offered completely digested protein or amino acids instead of unchanged protein. This was shown to hold good for the organism of the dog (*1*), and, generally speaking, undoubtedly holds true for the animal organism. In this connection, it may not be amiss to mention that the various amino acids perform special functions in the body. Thus, the amino acids lysin and cystin have been recognized by Abderhalden (*1*), Osborne and Mendel (*32, 33, 34, 35, 36*) as necessary for the function of growth, and in this capacity can not be replaced by any other amino acids. Equally, the diamino acids histidin and arginin (*1, 2*) are indispensable, since when they are removed from a complete amino acid mixture (obtained by protein hydrolysis), the remaining amino acids can no longer maintain the body in nitrogen equilibrium. Glycocoll (*1*), on the other hand, is not a necessary amino acid for the reason that it can be built up synthetically in the animal organism.

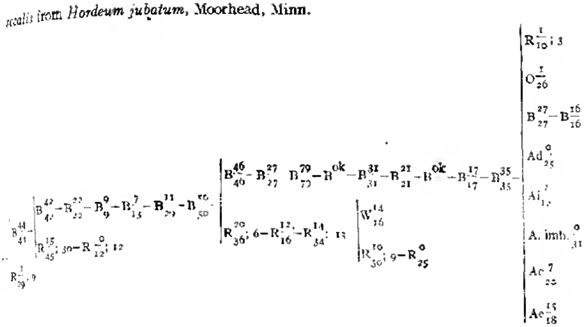
EXPERIMENTAL WORK

The spinach materials used in this investigation were secured in part from farms near Norfolk, Va.[1] Since some of these spinach materials (which were well mixed and treated as one lot) were not quite free from aphids, it was thought advisable to obtain samples free from the insects. These were taken from beds on another farm on May 6, 1916.[2] Three kinds of samples of the diseased and healthy plants, respectively, were prepared—namely, samples of the entire plant, of the tops, and of the roots. The samples were first fumigated with a tobacco preparation, then partly dried in the greenhouse for about a week, and partly in the electric drying oven at 40° to 50° C. The dried materials were ground, passed through a 40-mesh sieve, and kept in covered jars. As it was very soon discovered that the moisture content of the materials was changing, and also as it seemed more convenient to make practically all of the experiments with air-dried spinach, care was taken to make the ground spinach materials thoroughly air-dry. For this purpose they were exposed to the air in thin layers, in a place free from dust, until their moisture content became practically constant. The materials so prepared were kept in sealed jars ready for use. The results pertaining to the moisture content of the various spinach samples are expressed in Table I.

[1] By Dr. Rodney H. True, Dec. 1, 1915, and by the senior writer Jan. 21, 1916.

[2] By Mr. J. A. McClintock, then with the Virginia Truck Experiment Station.

…ecalis from ***Hordeum jubatum***, Moorhead, Minn.



January 23, 1917.

Ac=*Agropyron cristatum.*
Ad=*A. desertorum.*
Ae=*A. elongatum.*
A.imb.=*A. imbricatum.*
Ai=*A. intermedium.*
As=*A. smithii.*
A.sib.=*A. sibericum.*
At=*A. tenerum.*
Ap=*Alopecurus pratensis.*
Bp=*Bromus pumila.*
Hp=*Hordeum pusillum.*
Ar=*Agropyron repens.*
ok=100 per cent infection; record of exact number of infected leaves lost.
[1] Small uredinia.
[2] Plants died young.

TABLE I.—*Moisture content of air-dried healthy and diseased spinach*

HEALTHY SPINACH

No.	Date collected.	Kind of material.	Treatment of materials.	Quantity of air-dry substance used.	Water lost (at 100° C.). Observed.		Average.
				Gm.	*Gm.*	*Per cent.*	*Per cent.*
1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant....	Dried in electric drying oven at 50° C. for 48 hours.	0.8944	0.0346	3.87	3.86
2	....do.....	....do.....	....do.....	1.1323	.0436	3.85	
3	....do.....	....do.....	Remained in covered jars for about four weeks.	1.0000	.0437	4.37	4.31
4	....do.....	....do.....	....do.....	1.0000	.0426	4.26	
5	....do.....	....do.....	Exposed to air in thin layers for 24 hours.	.7653	.0371	4.85	4.84
6	....do.....	....do.....	....do.....	1.0338	.0499	4.83	
7	....do.....	....do.....	Exposed to air in thin layers for 24 hours longer.	.6197	.0318	5.10	5.10
8	....do.....	....do.....	Exposed to air in thin layers another 24 hours (total, 72 hours).	.9208	.0421	4.57	4.57
9	....do.....	....do.....	Remained in covered jars for about two weeks.	.8756	.0400	4.57	4.63
10	....do.....	....do.....	....do.....	.9272	.0436	4.70	
11	....do.....	Leaves.....	Dried in electric drying oven at 50° C. for 48 hours.	1.0000	.0372	3.72	3.78
12	....do.....	....do.....	....do.....	1.0000	.0383	3.83	
13	....do.....	....do.....	Remained in covered jars for about three weeks.	1.0000	.0416	4.16	4.17
14	....do.....	....do.....	....do.....	1.0000	.0419	4.19	
15	....do.....	....do.....	Exposed to air in thin layers for 48 hours.	.9731	.0484	4.97	4.97
16	....do.....	....do.....	Exposed to air in thin layers another 24 hours (total, 72 hours).	.8422	.0381	4.52	4.52
17	....do.....	....do.....	Remained in covered jars about two weeks.	.7850	.0349	4.45	4.45
18	....do.....	....do.....	....do.....	.9097	.0405	4.45	
19	.... do.....	Roots.....	Dried in electric drying oven at 50° C. for 48 hours.	1.0000	.0477	4.77	4.75
20	....do.....	....do.....	....do.....	1.0000	.0473	4.73	
21	....do.....	....do.....	Remained in covered jars for about three weeks.	1.0000	.0529	5.29	5.29
22	....do.....	.... do.....	....do.....	1.0000	.0528	5.28	
23	....do.....	....do.....	Exposed to air in thin layers for 48 hours.	.6825	.0349	5.11	5.11
24	....do.....	....do.....	Exposed to air in thin layers another 24 hours (total, 72 hours).	.9120	.0456	5.01	5.02
25	....do.....	....do.....	Remained in covered jars for about two weeks.	.8498	.0433	5.10	5.00
26	....do.....	....do.....	....do.....	.9164	.0448	4.89	
27	May 6, 1916....	Leaves.....	Exposed to air in thin layers for several days.	.9196	.0547	5.95	5.97
28	....do.....	....do.....	....do.....	1.0208	.0623	5.93	
29	....do.....	....do.....	....do.....	.6809	.0412	6.04	
30	....do.....	Roots.....	....do.....	.8288	.0593	7.15	7.12
31	....do.....	....do.....	....do.....	.6087	.0431	7.08	

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant....	Dried in electric oven at 50° C.	1.0000	0.0415	4.15	4.18
2	....do.....	....do.....	....do.....	1.0000	.0420	4.20	
3	....do.....	....do.....	Exposed to air in thin layers for 48 hours.	.8981	.0483	5.38	5.38
4	....do.....	....do.....	Exposed to air in thin layers for 24 more hours (total, 72 hours).	.8464	.0400	4.73	4.73
5	....do.....	....do.....	Remained in covered jars for about two weeks.	.8398	.0401	4.77	4.86
6	....do.....	....do.....	....do.....	1.1441	.0568	4.96	
7	....do.....	Leaves.....	Dried in electric oven at 50° C.	1.0000	.0582	5.82	5.82
8	....do.....	....do.....	....do.....	1.0000	.0580	5.80	
9	....do.....	....do.....	Exposed to air in thin layers 48 hours.	1.2732	.0704	5.53	5.53

TABLE I.—*Moisture content of air-dried healthy and diseased spinach*—Continued.

DISEASED SPINACH—Continued.

No.	Date collected.	Kind of material.	Treatment of materials.	Quantity of air-dry substance used.	Water lost (at 100° C.).		
					Observed.		Average.
				Gm.	*Gm.*	*Per cent.*	*Per cent.*
10	Dec. 1, 1915, and Jan. 21, 1916.	Leaves	Exposed to air in thin layers 24 more hours (total, 72 hours).	0.9967	0.0527	5.19	5.19
11	do	do	Remained in covered jars for about two weeks.	.9315	.0502	5.39	5.39
12	do	do	do	1.0003	.0558	5.58	5.48
13	do	Roots	Dried in electric oven at 50° C	1.0000	.0619	6.19	
14	do	do	do	1.0000	.0630	6.30	6.25
15	do	do	Exposed to air in thin layers for 48 hours.	.9747	.0464	5.07	5.07
16	do	do	Exposed to air in thin layers for 24 hours more (total, 72 hours).	.8112	.0445	5.49	5.49
17	do	do	Remained in covered jars for about two weeks.	.6920	.0410	5.92	5.93
18	do	do	do	.7512	.0447	5.95	
19	May 6, 1916	Leaves	Dried in electric oven at 50° C., which was followed by exposure of the substance to the air for several days.	.8313	.0532	6.40	6.27
20	do	do	do	.6923	.0426	6.15	
21	do	do	do	.9003	.0591	6.15	
22	do	do	do	.8570	.0545	6.36	
23	do	Roots	Treated like No. 19 to 22	.7860	.0533	6.78	6.76
24	do	do	do	.6100	.0411	6.74	

The examination of Table I reveals the fact that the drying of the spinach (entire plant) at 50° C. proceeded beyond the air-dry state, so that the moisture content of the material (which was kept in covered jars) increased from 3.86 to 4.31 per cent, and on exposure to the air in thin layers still further increased to 4.57 per cent. Further keeping of the material in covered jars showed that its moisture content remained practically constant, the small fluctuations being due undoubtedly to slight changes in the moisture of the air. The observations just mentioned hold good also for the spinach leaves and roots. The figures 4.63 per cent, the average for the entire plant, 4.45 per cent, average for the leaves, 5 per cent, average for the roots of the winter collection, and 5.97 and 7.12 per cent, respectively, for leaves and roots of the spring samples, were taken to represent the actual moisture percentages of the normal spinach materials in question, and were used in the calculation to the water-free basis of the results obtained in this investigation.

A glance at the second part of Table I shows that the samples of the entire plant of diseased spinach behaved very much like the healthy spinach, as far as the air drying is concerned. The diseased leaves and roots show a somewhat different behavior. Having been dried in the oven at 50° C. they still continued to decrease in their moisture content on exposure to the air in thin layers, which is evidently due to the fact that they had not been dried long enough in the oven. If not otherwise stated, the figures 4.86, 5.48, and 5.93 per cent for the whole plants, leaves, and roots, respectively, of the winter collection, and 6.27 and

6.76 per cent for the leaves and roots, respectively, of the spring collection, were used throughout this paper for calculating to the water-free basis the results obtained with the diseased materials in question

RELATION OF THE WATER CONTENT TO RETARDED GROWTH

The dwarfing effect of spinach-blight was shown in the weight of the plants used in a part of this work. Eighty diseased plants taken on the Jones farm weighed 552 gm., with an average weight of 6.9 gm. per plant. Forty-one healthy plants from the same beds weighed 647 gm., averaging 15.8 gm. per plant. The ratio of weights in disease and health was, therefore, 1 to 2.3. The leaves of the diseased plants were crisper, thicker in texture, and smaller in size than those of normally grown spinach. The root systems of the diseased plants were poorly developed, in comparison with those of the sound plants.

Considering the importance of water to the plant for the processes of transpiration, respiration, osmotic pressure, etc., it seemed worth while to make moisture estimations of the various plant tissues when in a fresh condition. Especially was it desirable to find out what difference, if any, there is between the healthy and the diseased spinach plants with regard to their moisture content. For this purpose several spinach samples were taken from various beds, immediately placed in air-tight jars, and the moisture estimations made within 24 hours. The results obtained are presented in Table II.

TABLE II.—*Moisture content of fresh healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Substance used.	Water lost (at 100°C.).		
				Observed.		Average.
			Gm.	*Gm.*	*Per cent.*	*Per cent.*
1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant	8. 5906	7. 2479	84. 37	85. 16
2	do	do	15. 2711	13. 1254	85. 95	
3	do	Leaves	7. 6908	6. 7502	87. 77	86. 28
4	do	do	7. 7867	6. 6009	84. 78	
5	May 6, 1916	Entire plant	12. 7295	10. 6611	83. 75	83. 75
6	do	Leaves	11. 5497	9. 6796	83. 81	83. 81
7	do	Roots	2. 1620	1. 6991	78. 59	78. 59

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant	7. 5448	6. 1100	80. 98	81. 55
2	do	do	5. 8979	4. 8691	82. 56	
3	do	do	28. 4032	23. 0374	81. 11	
4	do	Leaves	3. 4600	2. 9186	84. 35	83. 92
5	do	do	5. 4050	4. 5122	82. 48	
6	May 6, 1916	Entire plant	8. 0617	6. 4405	79. 89	79. 89
7	do	Leaves	7. 4214	5. 7435	77. 39	77. 39
8	do	Roots	1. 1746	0. 8963	76. 31	76. 31

When the two parts of the table are compared, it is readily seen that the healthy spinach samples, without exception, show a higher moisture content than the corresponding diseased samples. This is true not only of the entire plant, but of the leaves and roots as well. In other words, the spinach disease, physiologically characterized by a pronounced retardation of growth, is characterized chemically by a lower moisture content of its tissues. These data stand in full agreement with observations on certain animal tissues in disease and health. The water percentage of mouse carcinomata has been found by Cramer (*11*) to correspond with their rate of growth, the more rapidly growing tissues of the cancer showing a higher water content than the normal tissue, and vice versa. Evidently the rapidly growing plant cells like those of the cancer build up tissues with a comparatively high water content.

SPINACH NITROGEN

Since the main object was to ascertain the difference in quality and quantity of the nitrogenous compounds occurring in healthy spinach, on the one hand, and in diseased spinach, on the other, a number of nitrogen estimations were made according to the Gunning modification of Kjeldahl's method. As the moisture content of the spinach materials was not uniformly maintained it was necessary, in addition to nitrogen, also to run moisture estimations. The data which are recorded in Table III represent as a rule the average of two or more individual analyses.

TABLE III.—*Nitrogen content of healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Percentage of nitrogen found in oven-dried substance.					
1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant.	Feb. 4, 4.73	Mar. 2, 4.66	Mar. 14, 4.76	Mar. 16, 4.84	Mar. 17, 4.90	Mar. 28, 4.79
2	.....do.....	Leaves....	Feb. 12, 5.08	Mar. 2, 4.93	Mar. 16, 5.03	Mar. 17, 5.09	Mar. 28, 5.01	.....
3	.....do.....	Roots.....	Feb. 12, 3.80	Mar. 2, 3.72	Mar. 17, 3.82	Mar. 28, 3.88	.....	.....
4	May 6, 1916	Leaves....	May 13, 3.39	May 15, 3.40	.....	.....	.....	.....
5	.....do.....	Roots.....	May 15, 2.36	.....	.....	.....	.....	.....

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant.	Feb. 14–15, 3.59.	Feb. 17, 3.66	Mar. 15, 3.57	Mar. 16, 3.57	Mar. 17, 3.53	Mar. 29, 3.54
2	.....do.....	Leaves....	Feb. 14–15, 4.21.	Feb. 19, 4.13	Mar. 16, 4.31	Mar. 17, 4.31	Mar. 29, 4.22	.....
3	May 6, 1916	...do.....	May 13–15, 3.07.	.....	.....	.....	.....	.....
4	.....do.....	Roots.....	May 15, 2.74	.....	.....	.....	.....	.....

The examination of Table III (first part) reveals the fact that in the healthy plant the leaves have the highest and the roots the lowest nitrogen content, the figures for the entire plant lying between these two values. Likewise, by referring to Table III (second section) it may be

seen that in the diseased plant the nitrogen of the leaves is higher than that of either the roots or of the entire plant. A comparison of both sections further shows that the percentage of nitrogen in the healthy spinach (entire plant as well as leaves) is higher than in the corresponding diseased tissues, but that the nitrogen of the diseased roots by way of exception is somewhat higher than that of the healthy roots. If, as Boncquet (*6*) has claimed, denitrification sometimes takes place in diseased plant tissues whereby the nitrates are converted into nitrites and ammonia, the possibility of the ammonia escaping as such is not altogether out of the question. Such a proceeding would account for the smaller percentage of nitrogen in the diseased spinach.

PROTEIN NITROGEN OF THE SPINACH

The significance of protein as an integral constituent of protoplasm made it desirable to run a number of protein estimations of various healthy and diseased spinach tissues. The method applied here was originally proposed by Ritthausen (*39*) and perfected by Stutzer (*47*). One gm. of the finely powdered air-dry material was treated in a beaker with 100 cc. of water, heated to boiling, and kept on the steam bath for about 10 minutes. About 2 cc. of a concentrated potassium-alum solution were added, followed by 15 cc. of Stutzer's solution (corresponding to 0.45 gm. of copper hydroxid), and the whole was well stirred. On cooling, the insoluble residue was filtered off, washed with water, and the nitrogen estimated according to Kjeldahl's method. In a few instances, which will be mentioned, the following modification by Stutzer was used: To 1 gm. of the substance were added 100 cc. of absolute alcohol and 1 cc. of acetic acid, heated to the boiling point on a steam bath, and allowed to settle, when the supernatant liquid was carefully decanted through a filter. The substance which remained in the beaker was now treated with 100 cc. of water, heated to boiling, etc., as already outlined. The data obtained are summarized in Table IV.

While the modified method of Stutzer yields a somewhat higher percentage of protein nitrogen than the ordinary Stutzer method, it will be noticed by reference to the first section of Table IV that the proportion of protein nitrogen in leaves 8 to 10 is practically the same as in the roots 11 to 13. In other words, the protein nitrogen is practically uniform throughout the healthy spinach plant (see No. 1–7). As regards the actual quantities of protein nitrogen in the plants, as seen in the relation of protein nitrogen to dry weight, the leaves are considerably richer than the roots in both winter and spring samples. However, it will be noted that the spinach collected in May, 1916, shows a very much higher proportion of protein nitrogen than that collected in December, 1915, and January, 1916. The percentage of protein nitrogen calculated on dry weight is, however, greater in the plants of the earlier collection. The high relative percentage of protein nitrogen may perhaps be due to the fact that the winter samples were not as mature as those collected in May.

TABLE IV.—*Protein nitrogen of healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Date when estimation was made.	Protein nitrogen found.		
				Per cent of fresh weight.	Per cent of oven-dry weight.	Per cent of total nitrogen.
1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Mar. 13	0.356	2.40	51.96
2	do	do	do	.361	2.43	52.25
3	do	do	Apr. 1	.347	2.34	48.80
4	do	do	do	.344	2.32	48.42
	Average (1–4)			.352	2.37	50.26
5[a]	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Mar. 11	.370	2.49	53.44
6[a]	do	do	do	.365	2.46	52.79
7[a]	do	do	Apr. 8	.353	2.38	49.72
	Average (5–7)			.363	2.44	51.98
8	Dec. 1, 1915, and Jan. 21, 1916	Leaves	Apr. 1	.350	2.55	50.21
9	do	do		.346	2.52	49.77
	Average (8–9)			.348	2.54	49.99
10[a]	Dec. 1, 1915, and Jan. 21, 1916	Leaves	Apr. 8	.362	2.63	51.79
11[a]	do	Roots	Apr. 1		1.93	49.84
12[a]	do	do	do		1.89	48.25
	Average (11–12)				1.91	49.30
13[a]	Dec. 1, 1915, and Jan. 21, 1916	Roots	Apr. 8		2.94	52.49
14[a]	May 6, 1916	Leaves	May 17	.343	2.12	61.96
15[a]	do	do	do	.345	2.13	62.05
	Average (14–15)			.344	2.13	62.38
16	May 6, 1916	Roots	May 19	.319	1.49	63.28
17	do	do	do	.323	1.51	63.96
	Average (16–17)			.321	1.50	63.62

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Apr. 3	0.376	2.04	57.37
2	do	do	do	.371	2.01	56.81
	Average (1–2)			.374	2.03	57.09
3[a]	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Apr. 8	.384	2.08	58.30
4	do	Leaves	Apr. 3	.376	2.34	55.53
5	do	do	do	.370	2.30	54.45
	Average (4–5)			.373	2.32	54.99
6[a]	Dec. 1, 1915, and Jan. 21, 1916	Leaves	Apr. 8	.388	2.41	67.98
7	do	Roots	Apr. 3		2.54	61.38
8	do	do	do		2.50	60.37
	Average (7–8)				2.52	60.87
9[a]	Dec. 1, 1915, and Jan. 21, 1916	Roots	Apr. 8		2.52	62.84
10	May 6, 1916	Leaves	May 17	.427	1.89	62.43
11	do	do	do	.424	1.83	59.45
	Average (10–11)			.421	1.89	60.44
12	May 6, 1916	Roots	May 19	.450	1.90	69.3
13	do	do	do	.450	1.90	69.2
	Average (12–13)			.450	1.90	69.2

[a] Modified Stutzer's method.

Table IV, second part, shows that the protein nitrogen when referred to the total nitrogen in the diseased spinach is not distributed uniformly, the percentage in the roots being higher than that in the leaves. Here the samples collected in December and January have a considerably lower proportion of protein nitrogen referred to total nitrogen than the sample gathered in May. When referred to the dry weight of the plant material, the protein nitrogen of the roots exceeds that in the leaves in both winter and spring collections, the quantity present in the spring samples being clearly less than in those taken in winter. In this latter respect the diseased plants differ from the normal ones. The greater proportion of protein nitrogen to total nitrogen may indicate that the spring sample was in a riper state than the former winter sample.

When the first section of Table IV is compared with the second section, it is seen that the diseased spinach is not only able to build up protein but, with one exception, shows even a higher percentage of protein nitrogen (calculated on total nitrogen) than the healthy spinach, this being true of the leaves, the roots, and the entire plant. In case the protein nitrogen is related to the dry weight of the plant tissues, the situation is reversed as regards the entire plant and the leaves, the roots only showing a higher content in the diseased plants. This is true for both winter and spring material. So far as animal tissues are concerned, it was shown by Cramer (*11*) that certain rapidly growing cells and tissues build up protoplasm with less complex organic compounds (like proteins, etc.) than more slowly growing tissues.

From the data here presented it would seem that in spinach collected both in winter and spring the actual number of grams of protein nitrogen is greater in a given dry weight of healthy tops than in a like quantity of diseased material, this relation being plainly reversed in the case of the roots. It also seems clear that of the total nitrogen content a greater percentage is in protein form in the diseased than in the normal plants, the case of the leaves in the spring material being the only exception. It is further indicated that all winter material, diseased and healthy, is somewhat richer in protein nitrogen than the corresponding material collected in the spring.

NONPROTEIN NITROGEN OF THE SPINACH

The nonprotein nitrogen is usually found by subtracting the protein nitrogen from the total nitrogen. It seemed, however, desirable to make direct estimations of the nonprotein nitrogen as a check on the protein determinations. We proceeded as follows: The combined filtrate and washings from the copper precipitate, as obtained in the protein estimation according to Stutzer's method, were usually acidulated, concentrated on the water bath, quantitatively transferred to a Kjeldahl flask, and the nitrogen estimated as usual according to the Gunning modification of Kjeldahl's method. The results obtained are reported in Table V.

TABLE V.—*Nonprotein nitrogen of healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Date when estimation was made.	Nonprotein nitrogen found.			Nonprotein nitrogen calculated (difference from 100). Per cent of total nitrogen.
				Per cent of fresh weight.	Per cent of oven-dry weight.	Per cent of total nitrogen.	
1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	March 13	0.280	1.89	40.5	48.44
2	do	do	do	.280	1.89	40.4	47.18
3	do	do	April 1	Lost.			51.20
4	do	do	do	.331	2.23	46.65	51.58
	Average (1–4)			.297	2.00	42.52	49.35
5	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	March 22	.286	1.94	41.69	46.58
6	do	do	do	.282	1.90	40.75	47.27
7	do	do	April 8	.303	2.04	43.69	50.28
	Average (5–7)			.291	1.96	41.72	48.04
8	Dec. 1, 1915, and Jan. 21, 1916	Leaves	April 1	.333	2.28	45.02	49.79
9	do	do	do	.303	2.21	43.58	50.23
	Average (8–9)			.308	2.25	44.30	50.01
10	Dec. 1, 1915, and Jan. 21, 1916	Leaves	April 8	.285	2.08	41.22	48.22
11	do	Roots	April 1	Lost.			50.18
12	do	do	do		1.90	48.02	51.25
	Average (11–12)				1.90	48.02	50.72
13	Dec. 1, 1915, and Jan. 21, 1916	Roots	April 8		1.72	44.38	41.10
14	May 6, 1916	Leaves	May 17	.212	1.31	36.66	37.10
15	do	do	do	.198	1.22	35.82	37.25
	Average (14–15)			.205	1.27	37.24	37.43
16	May 6, 1916	Roots	May 19	.161	.75	31.75	36.12
17	do	do	do	.161	.75	31.75	36.04
	Average (16–17)			.161	.75	31.75	36.38

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	April 3	0.286	1.55	43.76	40.43
2	do	do	do	.288	1.56	44.18	43.17
	Average (1–2)			.287	1.56	43.97	42.80
3	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	April 8	.274	1.47	41.48	41.30
4	do	Leaves	April 3	.307	1.91	45.22	44.45
5	do	do	do	.293	1.83	43.27	45.55
	Average (4–5)			.301	1.87	44.25	44.91
6	Dec. 1, 1915, and Jan. 21, 1916	Leaves	April 8	.288	1.70	42.59	42.90
7	do	Roots	April 3		1.64	39.65	38.62
8	do	do	do		1.66	40.16	39.82
	Average (7–8)				1.65	39.91	39.13
9	Dec. 1, 1915, and Jan. 21, 1916	Roots	April 8		1.63	39.29	39.16
10	May 6, 1916	Leaves	May 17	.274	1.25	39.3	38.57
11	do	do	do	.274	1.21	39.3	40.53
	Average (10–11)			.274	1.21	39.3	39.16
12	May 6, 1916	Roots	May 19	.199	.84	30.49	30.7
13	do	do	do	.194	.82	29.98	30.8
	Average (12–13)			.197	.83	30.24	30.8

Examination of Table V, first section, shows that the calculated percentage of nonprotein nitrogen is usually higher than that found directly by analysis. This is especially marked in the case of No. 1 to 10, in which the filtrates from protein copper precipitate were concentrated on the water bath without having previously been acidulated. However, where acidulation of the filtrates did take place, as in No. 11 to 17, the difference in the results is still not inconsiderable. It seems reasonable to ascribe the loss of nonprotein nitrogen, at least in part, to the heating of the spinach with copper hydroxid (incidental to the Stutzer method) whereby the amids, which, as will be shown later, are contained in the spinach, undoubtedly lose a part of their nitrogen as ammonia in addition to the loss of ammonia present as such in the plant. From the data, which will be given later in this paper, it will be noticed that the sum of ammoniacal and acid amide nitrogen in the healthy spinach tissues is, as a rule, higher than the percentage present in the diseased tissues. For this reason it could be expected that the difference between the nonprotein nitrogen found and the nonprotein nitrogen calculated would be greater in the case of the healthy plant tissues. Just why in the case of the diseased spinach the nonprotein nitrogen calculated and found is practically the same needs still further investigation.

EXTRACTION OF THE NONPROTEIN NITROGEN OF THE SPINACH

A preliminary experiment was made to ascertain how the nitrogen can best be extracted from the spinach. Fifteen gm. of air-dry healthy spinach were treated in a round-bottom flask with 100 cc. of boiling hot ammonia-free water and digested on the steam bath with frequent shaking for 15 minutes, when the substance was filtered through a Buchner funnel provided with a filter, the extraction being repeated three more times. The final cake remaining on the Buchner funnel was thoroughly washed with boiling hot water. The combined filtrates and washings were acidified with acetic acid, boiled for a few minutes, filtered, and washed as usual on a filter. The filtrate and washings were cooled and made up to 1 liter. Two more portions of 15 gm. each were treated as outlined, with the difference that these two portions were extracted six and eight times, respectively. Nitrogen estimations in aliquots of the three extracts, showed that they contained, respectively, 55.86, 58.28, and 59.31 per cent of the total nitrogen. Inasmuch as the healthy spinach contains about 50 per cent of protein nitrogen (see Table IV), the fact that the three extracts contained nitrogen in excess of what could be expected, and more of it the more frequently the substance was extracted, seemed to indicate that a part of the protein nitrogen went into solution, probably through peptonization. The extraction was then modified so as to use a smaller amount of water and to effect the extraction more rapidly. The procedure was as follows:

Eighty gm. of air-dried spinach material were introduced into two 500 cc. round-bottom flasks (about equal amounts), and 200 cc. of boiling hot ammonia-free water added to each of the flasks, which were now kept on the water bath for 10 to 15 minutes. The digested spinach was then sucked off through a Buchner funnel, provided with a linen cloth filter (instead of a paper filter which filters very slowly). The cake remaining on the Buchner funnel was transferred to the round-bottom flasks, hot water added to original volume (about 150 cc. water to each flask), kept on the steam bath, etc., the extraction having been effected altogether four times. The combined extracts were distinctly acidified with acetic acid, using a small excess of it, and boiled for a few minutes. The extracts so treated were then filtered and washed on a Gooch crucible provided with a paper-pulp filter which, as was shown by Jodidi and Kellogg, proved to be an efficient filter not only for the estimation of phosphoric acid (*25*), calcium and magnesium (*26*), and in general for quantitative analysis (*27*), but also for the separation of solids from liquid (*28*) in general chemical work, especially when a comparatively small precipitate is contained in a large volume of liquid. On cooling, the liquid was made up to 2,000 cc., of which two or three portions of 25 cc. each were oxidized according to Kjeldahl's method to ascertain the amount of nitrogen extracted. The data are summarized in Table VI.

TABLE VI.—*Nitrogen in water extract of healthy and diseased spinach*

No.	Date when spinach was collected.	Kind of material.	Nitrogen found.			
			Per cent of fresh weight.	Per cent of oven-dry weight.	Per cent of total nitrogen.	Nitrogen in 1 cc. of extract.
						Mgm.
1	Dec. 1, 1915, and Jan. 21, 1916.	Healthy spinach (entire plant).	0.375	2.53	52.85	0.9656
2	.....do.....	Healthy leaves.....	.353	2.57	50.64	.9812
3	.....do.....	Diseased spinach (entire plant).	.343	1.86	52.40	.7069
4	.....do.....	Diseased leaves.....	.357	2.22	52.72	.8412
5	May 6, 1916.....	.....do.....	.352	1.56	50.74	.5840

A glance at Table VI shows that under the conditions outlined the proportions of nitrogen extracted by water from the various spinach materials were fairly uniform, this being true of both the healthy and the diseased plants. When we further compare No. 1 and 2 of Table VI with No. 1 and 13 of Table IV, first section, we find that the total sum of the water-soluble nitrogen and the protein nitrogen is from 1 to 2 per cent above 100, this being undoubtedly due to the fact that the Stutzer method ordinarily yields a somewhat too high percentage of protein nitrogen. The discrepancy is, however, greater in the case of the diseased

spinach, where the amounts of soluble nitrogen plus protein nitrogen range from 106 to 110 per cent. Giving due allowance for the inaccuracies of the operation involved, it is reasonable to ascribe the discrepancies noticed, not merely to the high results of Stutzer's protein method, but also to the possibility that the protein of the diseased spinach examined may perhaps differ from that of the healthy spinach in undergoing changes more readily.

DISTRIBUTION OF THE WATER-SOLUBLE NITROGEN IN SPINACH

The determination of the nitrogen of acid amids, diamino acids, and monoamino acids was made essentially according to Hausmann's method (*19*, *20*), as modified by Osborne and Harris (*31*), and as applied to soils by one of the writers (*Jodidi 21*, *22*, *23*, *24*). The estimation of the nitrogen of compounds other than those mentioned was made according to methods which will briefly be described subsequently in this paper.

Ordinarily to 250 cc. of spinach solution, prepared as outlined above and corresponding to 10 gm. of air-dry spinach, concentrated hydrochloric acid was added to a concentration of 20 per cent, and boiled under a reflux condenser 30 minutes. The hydrolyzed substance was now quantitatively transferred to a porcelain dish and evaporated on the steam bath practically to dryness.

In order to ascertain whether or not all of the acid amid nitrogen was split off as ammonia under the conditions outlined, and at the same time to completely hydrolyze any polypeptids present, another portion of 250 cc. of the same spinach extract was treated with enough concentrated hydrochloric acid to give a 20 per cent concentration and boiled under a reflux condenser for 8 hours. The fact that not all of the nitrogenous constituents of plants are known, and the consideration that some of them might be decomposed by boiling with 20 per cent hydrochloric acid, made it desirable to hydrolize the spinach extract with as dilute an acid as possible, but strong enough to split off quantitatively in the form of ammonia the nitrogen of acid amids present. Inasmuch as asparagin and glutamin are probably the principal acid amids contained in plants, their behavior toward hydrochloric acid of different strength was here of special interest. Unfortunately, the writers had to confine the experiment to asparagin only, not having any glutamin. Its chemical behavior, however, is known to be very similar to that of asparagin, its lower homolog.

Two and five-tenths gm. of crystallized asparagin, with a nitrogen percentage of 18.38 (the formula $C_4H_8N_2O_3 + H_2O$ requires 18.66 per cent N), were dissolved in water and made up to 350 cc. Of this solution portions of 20 cc., each, were transferred to small round-bottom flasks to which concentrated hydrochloric acid was added until the desired per-

centage was obtained, and boiled under reflux for a definite time. No. 1 and 2 were then neutralized with sodium hydroxid, and on addition of 3 gm. of magnesium oxid were subjected to distillation, while No. 3 to 14 were directly distilled with 3 gm. of magnesia. The results are recorded in Table VII.

TABLE VII.—*Hydrolysis of asparagin with hydrochloric acid*

No.	Strength of hydrochloric acid.	Boiled under reflux.	Ammoniacal nitrogen found.	
			Observed.	Average.
	Per cent.	*Hours.*	*Mgm.*	*Per cent.*
1	20	½	12.48	.....
2	20	½	12.48	47.53
3	2	1	11.43	.....
4	2	1	11.50	43.68
5	3	1	Lost.	.....
6	3	1	11.61	44.22
7	4	1	12.34	.....
8	4	1	12.05	46.46
9	2	2	11.92	.....
10	2	2	12.22	45.97
11	3	2	12.27	.....
12	3	2	12.36	46.92
13	4	2	12.36	.....
14	4	2	12.44	47.22

From the table it follows that boiling asparagin with 4 per cent hydrochloric acid for 2 hours split off, in the form of ammonia, as much nitrogen as did boiling with 20 per cent hydrochloric acid for 30 minutes.

DESCRIPTION OF METHODS

(1) THE TOTAL SOLUBLE NITROGEN, as mentioned already, was usually estimated in 25-cc. portions of the aqueous spinach extract according to the Gunning modification of the Kjeldahl method.

(2) THE NITROGEN OF AMMONIA present as such in the spinach materials was estimated according to Grafe's method (*14*), which is based upon the work of Wurster, Boussignault, Folin, Krüger, and Reich. Ordinarily 20 gm. of air-dry spinach were introduced into a 2-liter round-bottom flask with the aid of 50 cc. of saturated sodium-chlorid solution, 50 cc. of distilled water, 25 cc. alcohol, and the whole mixed thoroughly. The flask was then connected with a Péligot tube (of about 400 cc. capacity) usually containing 20 cc. of *N*/5 sulphuric acid, whereupon 25 cc. of saturated sodium-carbonate solution were added. After the whole apparatus was carefully made air-tight, the burner under the water bath was lighted and the suction pump (May-Nelson) brought into action. The first 3 hours the distillation took place at 25° to 28° C., the last 3 or 4 hours at about 37°. The absolute pressure observed was mostly about 20 mm. (ranging from 5 to 45 mm.). The ammonia found in the spinach by this method was taken to represent also the ammonia in the aqueous spinach extract. A direct ammonia determination in the spinach extract, because of the heat applied at the extraction, was deemed inaccurate.

(3) THE ACID AMIO NITROGEN was estimated in 250-cc. portions of the spinach extract. The latter, on hydrolysis, was evaporated to dryness, transferred quantitatively to an 800-cc. Kjeldahl flask of Pyrex glass with the aid of 100 cc. of distilled

water. Two grams of magnesium oxid, previously reduced to cream with 100 cc. of water, were added to the flask and distilled, the distillate being received in an Erlenmeyer flask containing *N/10* sulphuric acid. From the ammoniacal nitrogen found by titration the ammonia nitrogen found in (2) was subtracted, giving the nitrogen of the acid amids.

That the distillates obtained at the distillation of the hydrolyzed and evaporated spinach extracts with magnesium oxid actually represented ammonia was shown by the preparation of chloroplatinates from the distillates in question. The platinum double salts usually, though not always, showed a platinum percentage which was close to the 43.93 required by the formula $(NH_4)_2PtCl_6$.

(4) THE HUMIN NITROGEN, which resulted from the action of the boiling hydrochloric acid upon the aqueous spinach, and which was due in part to the presence in the latter of diamino acids and monoamino acids, was estimated in the magnesium-oxid residue remaining in the Kjeldahl flask from the acid amid estimation in (3). The magnesium-oxid residue was completely decanted off on a Gooch crucible provided with a linen cloth filter, the filtrate being received in a beaker placed in Witt's filtering apparatus. The residue was now repeatedly (about 10 times) treated with small quantities (25 cc.) of boiling hot water, and finally quantitatively filtered and washed on the Gooch crucible (filtrates and washings being received in another beaker). The magnesium-oxid cake with the aid of dilute sulphuric acid was then quantitatively transferred to a 500 cc. Kjeldahl flask and the nitrogen estimated according to Kjeldahl's method.

(5) THE BASIC NITROGEN was estimated in the filtrates and washings from the magnesium-oxid cake obtained in (4). The combined liquids, with the exception of the first decantation, which in order to avoid the formation of a brown precipitate was not concentrated, were evaporated on the water bath to a small volume. This was added to the first decantation, cooled to 20° C., made up to 100 cc., and treated with 5 gm. of sulphuric acid, 30 cc. of a solution containing 20 gm. of phosphotungstic acid, and 5 gm. of sulphuric acid per 100 cc. After at least 24 hours, the precipitate was filtered through an S. and S. filter and washed with about 200 cc. of a solution containing 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 cc., the washing being effected by rinsing the precipitate from the filter into a beaker and returning to the filter three times. The washed precipitate was then Kjeldahlized and tritrated, giving the proportion of the basic nitrogen.

That diamino acids formed a part of the basic nitrogen was demonstrated in the following way: Another portion of the phosphotungstic-acid precipitate obtained in the manner outlined was treated with barium hydroxid in excess, and the barium phosphotungstate filtered out and washed. Filtrate and washings were not treated with carbon dioxid to remove the excess of baryta, the filtrate and washings from barium carbonate being evaporated on the water bath to a small volume. This concentrated solution gave the following tests:

1. Phosphotungstic-acid solution gave immediately a heavy, white precipitate.
2. Phosphomolybdic acid gave a yellow precipitate.
3. Mercuric chlorid gave a gray flocculent precipitate.
4. Silver nitrate gave a grayish-white precipitate, soluble in excess of ammonia.
5. The solution was distinctly alkaline.
6. Addition of neutralized formaldehyde to the alkaline solution made it turn acid, pointing to the presence of carboxyl and amino groups.

(6) THE MONOAMINO ACID NITROGEN was estimated in the filtrate from the phosphotungstic-acid precipitate. To remove the excess of phosphotungstic and sulphuric acids from the filtrate the latter was treated with barium hydroxid whose excess was removed with carbon dioxid. Both the barium phosphotungstate (plus barium sulphate) and the barium-carbonate precipitates were repeatedly washed with boiling

hot water. Filtrate and washings from barium carbonate were evaporated on the water bath to small volume, filtered, washed, and finally made up to 100 cc., of which 20 cc. were Kjeldahlized to ascertain the quantity of nitrogen present. The remaining 80 cc. were formol-titrated, having previously been freed from carbon dioxid and phosphoric acid whose presence would interfere with formol titration (*46*). For this purpose there were added to the 80 cc. of the solution 2 gm. of barium chlorid which were dissolved by shaking, then 1 cc. of a 0.5 per cent of phenolphthalein solution and enough of saturated barium hydroxid solution until a red color appeared. Five cc. of *N*/5 barium hydroxid were added in excess, made up to the mark with water (usually 100 cc.), shaken and filtered after a few minutes. Of this filtrate definite quantities, usually of 40 cc. each, were neutralized with *N*/5 hydrochloric acid and formol titrated, the data of the titration being recalculated to the total soluble nitrogen. In cases in which the solution was too dark for formol titration, it was decolored by the formation in the solution of a precipitate of silver chlorid (or copper sulphid). Ordinarily the solution was rendered acid with *N*/2 hydrochloric acid, whereupon about 10 cc. of *N*/2 silver-nitrate solution was gradually added while the flask was constantly shaken. Inasmuch as the presence of silver would interfere with the formol tritration, care was taken to insure an excess of the chlorin ion in the solution by adding to it about 5 cc. of *2 N* barium-chlorid solution. The silver-chlorid precipitate formed in the solution usually carries down the coloring substance, so that the filtrate shows a yellowish light color and can then readily be formol titrated.

(7) THE PEPTID NITROGEN was estimated in the aqueous spinach extract, on hydrolysis with 20 per cent hydrochloric acid for 8 hours. From the hydrolyzed solution ammonia and humin nitrogen were removed in the manner already described. The filtrate and washings from magnesium-oxid residue were then evaporated on the water bath, cooled, and made up to 100 cc., of which 20 cc. were Kjeldahlized to ascertain the nitrogen present. The remaining 80 cc. were freed from carbon dioxid, phosphoric acid, and coloring matter, as outlined above. Aliquots of the filtrate (made up to 100 cc.) usually portions of 40 cc. each, were then formol-titrated. From the amino acid nitrogen found here, was subtracted the amino-acid nitrogen (minus the ammonia present as such) which was found directly in the water extract of the spinach materials by formol titration.

(8) THE RESIDUAL SOLUBLE NITROGEN made up of nitrogenous compounds other than those given above constitutes the difference between the total water-soluble nitrogen and the sum of the nitrogen found as ammoniacal nitrogen in (2), acid amid nitrogen in (3), humin nitrogen in (4), basic nitrogen in (5), monoamino acid nitrogen in (6), and peptid nitrogen in (7).

The results obtained by the methods described are summarized in Table VIII.

As will be seen, the first section of Table VIII presents the results expressed in percentage of the total soluble nitrogen of the spinach materials, while in the second and third sections the data are expressed in percentage of the total nitrogen and of the oven-dried spinach material, respectively.

The total nitrogen of the spinach is given in Table III. The examination of the latter part of this table shows that, with one exception,[1] the nitrogen content of all the healthy spinach materials is higher than the nitrogen content of the corresponding diseased materials. It is true that investigation showed that the soil of the diseased spinach has a

[1] Compare No. 5 of Table III, first part, with No. 4 of the second part.

somewhat higher concentration of salts than the soil of the healthy plants; other properties, however, like humus content, water-holding capacity, were in favor of soil poorer in nitrogen. If any clear difference existed, the soil of the diseased spinach was superior to that yielding the healthy spinach. This being true, it was evident that the cause of the lower nitrogen content of the diseased spinach was to be sought in the plant itself.

The loss of nitrogen may occur through denitrification, which, if it does take place in the diseased spinach, would satisfactorily explain its lower nitrogen content as well as other phenomena which will be mentioned subsequently in this paper. The first step in the process of denitrification consists primarily in the reduction of the nitrates to nitrites. The latter react on acid amids, which, as we have seen, are present in the spinach tissues, whereby elementary nitrogen is set free. This reaction can be presented chemically as follows:

$$\underbrace{R.CO.NH_2}_{\text{Acid amid.}}+NO.OH=\underbrace{R.CO.OH}_{\text{Fatty acid.}}+N_2+H_2O.$$

Thus, both the nitrogen of nitrates and of acid amids would be lost through the process of denitrification, explaining the lower nitrogen content in the diseased spinach.

In the process of denitrification the reduction seems not to stop after the nitrates have been reduced to nitrites, but the latter seem often in part to be further reduced to ammonia. If this took place, it is evident that the diseased spinach tissues would show a somewhat higher percentage of ammoniacal nitrogen than the corresponding healthy tissues. This is actually the case, as a glance at the data from the winter-collected material in Table VIII shows (column 7).

Conversely, a somewhat smaller acid amid nitrogen content would be expected in the diseased spinach tissues than in the corresponding healthy tissues, because of the fact that the acid amids by reacting with the nitrites in accordance with the supposed reaction would lose their nitrogen in gas form. Evidence to support this explanation is found in Table VIII (column 8).

An examination of column 9 of the same table shows that the percentage of humin nitrogen in the diseased spinach is, as a rule, higher than in the healthy spinach. Hart and Bentley (*15*) and Roxas (*40*) in Hart's laboratory have demonstrated that the formation of humin nitrogen takes place at the expense of diamino acids and monoamino acids. Inasmuch as, under similar conditions, the humin nitrogen was formed in the spinach extract by boiling with hydrochloric acid, it is evident that the proportions of monoamino acids and basic nitrogen originally present were higher than the values given in Table VIII (columns 10 and 11)—namely, by the amount of humin nitrogen formed

TABLE VIII.—*Distribution of the water-soluble nitrogen in spinach*

RESULTS EXPRESSED IN PERCENTAGE OF THE TOTAL SOLUBLE NITROGEN OF THE SPINACH MATERIALS

No.	Date when spinach was collected.	Spinach material.	Strength of hydrochloric acid.	Time of digest.	Total nitrogen (H_2O soluble).	Ammonia nitrogen.	Acid amid nitrogen.	Humin nitrogen.	Basic nitrogen.	Mono-amino acid nitrogen.	Peptid nitrogen.	Residual soluble nitrogen.
			Per cent.	*Hours.*								
1	Dec. 1, 1915, and Jan. 21, 1916	Healthy, entire plant	4	2	100	6.09	13.25	5.73	25.95	18.74	.....	.....
2	....do	....do	20	½	100	6.09	16.32	6.46	24.44	20.67	.....	.....
3	....do	....do	20	8	100	6.09	22.19	4.07	.....	.....	4.66	17.34
4	....do	Healthy leaves	20	8	100	4.82	22.69	5.41	.....	.....	7.48	10.56
5	....do	....do	20	11	100	4.82	23.92	5.01	.....	.....	.....	.....
6	....do	....do	20	½	100	4.82	17.77	5.23	30.12	18.95	.....	.....
7	....do	....do	4	2	100	4.82	14.21	7.51	29.84	19.40	.....	.....
8	....do	....do	20	½	100	4.82	16.38	7.17	27.42	19.13	.....	.....
9	....do	....do	4	2	100	4.82	13.63	6.42	28.06	18.09	.....	.....
10	....do	Diseased, entire plant	4	2	100	8.14	13.53	10.53	27.47	18.83	.....	.....
11	....do	....do	20	½	100	8.14	14.77	7.06	27.79	22.07	.....	.....
12	....do	....do	20	8	100	8.14	17.56	5.76	.....	.....	3.21	15.47
13	....do	Diseased leaves	4	2	100	7.78	13.48	9.35	26.45	17.11	.....	.....
14	....do	....do	20	½	100	7.78	13.88	10.00	26.79	21.82	.....	.....
15	....do	....do	20	8	100	7.78	17.41	5.44	.....	.....	1.77	18.99
16	May 6, 1916	....do	4	2	100	3.88	11.51	13.04	26.12	24.63	.....	.....
17	....do	....do	20	½	100	3.88	10.74	Lost.	18.89	.....	.....	.....
18	....do	....do	20	8	100	3.88	13.23	9.51	.....	.....	5.09	27.54

RESULTS EXPRESSED IN PERCENTAGE OF THE TOTAL NITROGEN OF THE SPINACH MATERIALS

1	Dec. 1, 1915, and Jan. 21, 1916	Healthy entire plant	4	2	52.85	3.22	7.00	3.03	13.71	9.90	.....	.....
2	....do	....do	20	½	52.85	3.22	8.62	3.41	12.91	10.89	.....	.....
3	....do	....do	20	8	52.85	3.22	11.72	2.47	.....	.....	2.47	9.17
4	....do	Healthy leaves	20	8	50.64	2.55	11.37	2.74	.....	.....	3.79	5.34
5	....do	....do	20	11	50.64	2.55	12.00	2.54	.....	.....	.....	.....
6	....do	....do	20	½	50.64	2.55	8.89	2.63	15.25	9.60	.....	.....
7	....do	....do	4	2	50.64	2.55	7.09	3.80	15.11	9.82	.....	.....
8	....do	....do	20	½	50.64	2.55	9.20	3.63	13.88	9.69	.....	.....
9	....do	....do	4	2	50.64	2.55	6.79	3.25	14.21	9.26	.....	.....
10	....do	Diseased entire plant	4	2	52.40	4.30	7.06	5.52	14.39	9.87	.....	.....
11	....do	....do	20	½	52.40	4.30	7.70	3.79	14.56	11.56	.....	.....
12	....do	....do	20	8	52.40	4.30	9.17	3.02	.....	.....	1.68	8.12
13	....do	Diseased leaves	4	2	52.72	4.11	7.10	4.95	13.95	9.02	.....	.....
14	....do	....do	20	½	52.72	4.11	7.31	5.27	14.12	11.50	.....	.....

15	....do	....do	20	8	52.72	4.11	9.17	2.87	.....	.....	0.93	10.02
16	May 6, 1916	....do	4	2	50.74	2.05	5.33	0.28	12.53	7.42	.....	.....
17	....do	....do	20	1/5	50.74	2.05	4.96	Lost.	9.06	.....	.....	.....
18	....do	....do	20	8	50.74	2.05	6.16	4.56	.....	.....	2.59	15.43

RESULTS EXPRESSED IN PERCENTAGE OF OVEN-DRIED SPINACH MATERIALS

1	Dec. 1, 1915, and Jan. 21, 1916	Healthy entire plant	4	2	2.53	0.154	0.330	0.15	0.66	0.47	.....	.....
2	....do	....do	20	1/5	2.53	.154	.416	.16	.62	.52	.....	.....
3	....do	....do	20	8	2.53	.154	.566	.12	.....	.....	0.127	0.433
4	....do	Healthy leaves	20	8	2.57	.129	.581	.14	.....	.....	.195	.265
5	....do	....do	20	11	2.57	.129	.611	.13	.....	.....	.....	.....
6	....do	....do	20	1/5	2.57	.129	.441	.13	.77	.49	.....	.....
7	....do	....do	4	2	2.57	.129	.361	.19	.72	.50	.....	.....
8	....do	....do	20	1/5	2.57	.129	.471	.18	.70	.49	.....	.....
9	....do	....do	4	2	2.57	.129	.341	.16	.72	.46	.....	.....
10	....do	Diseased entire plant	4	2	1.86	.152	.248	.20	.51	.35	.....	.....
11	....do	....do	20	1/5	1.86	.152	.268	.13	.52	.42	.....	.....
12	....do	....do	20	8	1.86	.152	.328	.11	.....	.....	.061	.279
13	....do	Diseased leaves	4	2	2.22	.174	.296	.21	.59	.38	.....	.....
14	....do	....do	20	1/5	2.22	.174	.306	.22	.60	.49	.....	.....
15	....do	....do	20	8	2.22	.174	.386	.12	.....	.....	.052	.398
16	May 16, 1916	....do	4	2	1.56	.063	.167	.19	.38	.23	.....	.....
17	....do	....do	20	1/5	1.56	.063	.157	.28	.....	.....	.....	.....
18	....do	....do	20	8	1.56	.063	.187	.14	.....	.....	.078	.482

As will be seen by reference to Table VIII, column 12, the percentage of peptid nitrogen is usually higher in the healthy tissues than in the diseased tissues.

The diseased spinach collected in May, 1916, is strikingly different from the diseased spinach gathered in December, 1915, and January, 1916. An examination of Table VIII shows that the percentage of nitrogen in the form of acid amids and basic nitrogen is smaller in the former than in the latter. This, together with the fact that the protein nitrogen of the May sample is higher than that of the December and January samples (see Table IV) points to the former as being in a riper state whereby the acid amids and basic-nitrogen compounds have preferably been used by the plant for building up protein substance.

The results for residual soluble nitrogen in which the different fluctuations of the various constituents (ammoniacal nitrogen, acid amid nitrogen, etc.) are reflected, range from 10 to 17 per cent in the case of the healthy spinach, and from 15 to 19 per cent in the case of the diseased materials. The May sample has as much as 27 per cent, calculated on the total soluble nitrogen.

SUMMARY

(1) It has been shown (p. 381–384) that carbohydrates accumulate in the leaves of plants affected with the spinach-blight in considerably greater quantity than in normal leaves.

(2) In this paper it is shown that the accumulation is not due to the inability of the diseased plants to make proteins. Although these constituents are found in the tops of the diseased plants in a somewhat smaller percentage calculated on the dry weight of the material than in the normal tops, the proteins make up a larger proportion of the total nitrogen in the diseased than in the healthy material. The proteins in the roots of diseased plants exceed those found in the roots of normal plants, both in reference to the dry weight of the material and to the total nitrogen.

(3) Spinach-blight is physiologically characterized by retarded growth, and a lower moisture content. This seems to be due to the fact that the rapidly growing normal tissues are comparatively rich in water and poor in complex organic compounds, such as proteins, etc.

(4) The lower percentage of total nitrogen and of acid amid nitrogen in the diseased material can best be explained by the assumption that denitrification takes place in those tissues, whereby a part of the nitrogen may be lost either as elementary nitrogen or in the form of ammonia.

(5) The reason for the higher proportion of ammoniacal nitrogen in the diseased material than in the corresponding healthy tissues would be sought in the processes of denitrification, whereby a part of the nitrites is further reduced to ammonia.

LITERATURE CITED

(1) ABDERHALDEN, Emil.
1915. WEITERE STUDIEN ÜBER DEN STICKSTOFFSTOFFWECHSEL . . . *In* Ztschr. Physiol. Chem., Bd. 96, Heft ½, p. 1–147.

(2) ACKROYD, Harold, and HOPKINS, F. G.
1916. FEEDING EXPERIMENTS WITH DEFICIENCIES IN THE AMINO-ACID SUPPLY; ARGININE AND HISTIDINE AS POSSIBLE PRECURSORS OF PURINES. *In* Biochem. Jour., v. 10, no. 4, p. 551–576, 3 fig. References, p. 576.

(3) BACH, A., Chodat, R.
1904. UNTERSUCHUNGEN ÜBER DIE ROLLE DER PEROXYDE IN DER CHEMIE DER LEBENDEN ZELLE. *In* Ber. Deut. Chem. Gesell., Jahrg. 37, No. 10, p. 2434–2440, 2 fig.

(4) BERTHELOT, and ANDRE, G.
1895. SUR LA PRÉSENCE DE L'ALUMINE DANS LES PLANTES ET SUR SA REPARTITION. *In* Compt. Rend. Acad. Sci. [Paris], t. 120, no. 6, p. 288–290.

(5) BERTRAND, Gabriel.
1897. SUR L'INTERVENTION DU MANGANÈSE DANS LES OXYDATIONS PROVOQUÉES PAR LA LACCASE. *In* Compt. Rend. Acad. Sci. [Paris], t. 124, no. 19, p. 1032–1055.

(6) BONCQUET, P. A.
1916. PRESENCE OF NITRITES AND AMMONIA IN DISEASED PLANTS . . . *In* Jour. Amer. Chem. Soc., v. 38, no. 11, p. 2572–2576.

(7) BUNZELL, H. H.
1912. THE MEASUREMENT OF THE OXIDASE CONTENT OF PLANT JUICES. U. S. Dept. Agr. Bur. Plant Indus. Bul. 238, 40 p., illus., 2 pl.

(8) ———
1913. A BIOCHEMICAL STUDY OF THE CURLY-TOP OF SUGAR BEETS. U. S. Dept. Agr. Bur. Plant Indus. Bul. 277, 28 p., tab.

(9) ———
1914. OXIDASES IN HEALTHY AND IN CURLY-DWARF POTATOES. *In* Jour. Agr. Research, v. 2, no. 5, p. 373–404. Literature cited, p. 403–404.

(10) ———
1914. A SIMPLIFIED AND INEXPENSIVE OXIDASE APPARATUS. *In* Jour. Biol. Chem., v. 17, no. 3, p. 409–411, 1 fig.

(11) CRAMER, W.
1916. ON THE BIOCHEMICAL MECHANISM OF GROWTH. *In* Jour. Physiol., v. 50, no. 5, p. 322–334, 2 fig.

(12) CZAPEK, Friedrich.
1905. BIOCHEMIE DER PFLANZEN. Bd. 2. Jena.

(13) DOBY, Géza.
1911–12. BIOCHEMISCHE UNTERSUCHUNGEN ÜBER DIE BLATTROLLKRANKHEIT DER KARTOFFEL. *In* Ztschr. Pflanzenkrank., Bd. 21, Heft ½, p. 10–17; Heft 6, p. 321–336, 1911; Bd. 22, Heft 4, p. 204–211; Heft 7, p. 401–403 1912. Literatur, Bd. 21, p. 17.

(14) GRAFE, Erich.
1906. METHODISCHES ZUR AMMONIAKBESTIMUNG IN TIERISCHEN GEWEBEN *In* Ztschr. Physiol. Chem., Bd. 48, Heft 3/4, p. 300–314.

(15) HART, E. B., and BENTLEY, W. H.
1915. THE CHARACTER OF THE WATER-SOLUBLE NITROGEN OF SOME COMMON FEEDING STUFFS. *In* Jour. Biol. Chem., v. 22, no. 3, p. 477-483.

(16) HARTER, L. L.
1909. THE CONTROL OF MALNUTRITION DISEASES OF TRUCK CROPS. Va. Truck Exp. Sta. Bul. 1, 16 p., 4 fig.

(17) HARTER, L. L.
1910. SPINACH TROUBLES AT NORFOLK AND IMPROVEMENT OF TRUCKING SOILS. Va. Truck Exp. Sta. Bul. 4, p. 61–80, 22 fig.

(18) HASSELBRING, Heinrich, and ALSBERG, C. L.
1910. STUDIES UPON OXIDASES. (Abstract.) *In* Science, n. s. v. 31, no. 799, p. 637.

(19) HAUSMANN, Walther.
1899. UEBER DIE VERTHEILUNG DES STICKSTOFFS IM EIWEISSMOLEKÜL. *In* Ztschr. Physiol. Chem., Bd. 27, Heft 1/2, p. 95–108.

(20) ———.
1900. UEBER DIE VERTHEILUNG DES STICKSTOFFS IM EIWEISSMOLEKÜL. II. Mittheilung. *In* Ztschr. Physiol. Chem., Bd. 29, Heft 2, p. 136–145.

(21) JODIDI, S. L.
1909. ORGANIC NITROGENOUS COMPOUNDS IN PEAT SOILS. Mich. Agr. Exp. Sta. Tech. Bul. 4, 28 p. 1909. Also in Jour. Amer. Chem. Soc., v. 32, no. 3, p. 396–410. 1910.

(22) ———
1911. THE CHEMICAL NATURE OF THE ORGANIC NITROGEN IN THE SOIL. Iowa Agr. Exp. Sta. Research Bul. 1, 46 p, 1 fig. 1916. Also in Jour. Amer. Chem. Soc., v. 33, no. 7, p. 1226–1241.

(23) ———
1912. THE CHEMICAL NATURE OF THE ORGANIC NITROGEN IN THE SOIL. *In* Jour. Amer. Chem. Soc., v. 34, no. 1, p. 94–99.

(24) ——— and WELLS, A. A.
1911. THE CHEMICAL NATURE OF THE ORGANIC NITROGEN IN THE SOIL. Influence of various factors on decomposition of soil organic matter. Iowa Agr. Exp. Sta. Research Bul. 3, p. 113–154, 4 fig.

(25) ——— and KELLOGG, E. H.
1916. A SIMPLE, EFFICIENT, AND ECONOMIC FILTER. *In* Biochem. Bul., v. 5, no. 18–19, p. 87–94. Also in Proc. 2d Pan Amer. Sci. Congr., v. 8 (sect. 7), p. 704–708, 1917. Reviewed in Rev. Gén. Sci., v. 27, p. 392, 1916.

(26) ——— ———
1916. THE APPLICATION OF THE PAPER PULP FILTER TO THE QUANTOTATIVE ESTIMATION OF CALCIUM AND MAGNESIUM. *In* Jour. Franklin Inst., v. 181, no. 2, p. 217–232, 1 fig. Also in Chemical Engineer, v. 23, p. 60–65; 1916; and in Proc. 2d Pan Amer. Sci. Congr., v. 8 (sect. 7), p. 843–849. 1917.

(27) ——— ———
1916. THE GENERAL APPLICABILITY OF THE PAPER PULP FILTER TO QUANTITATIVE ANALYSIS. *In* Jour. Indus. and Engin. Chem., v. 8, no. 4, p. 317–319.

(28) ———
1916. NOTE ON THE APPLICABILITY OF THE PAPER PULP FILTER TO THE SEPARATION OF SOLIDS FROM LIQUIDS. *In* Jour. Franklin Inst., v. 182, no. 1, p. 61–67. Also in Chem. Engin. and Manuf., v. 24, no. 2, p. 79–81. 1916.

(29) MCCLINTOCK, J. A., and SMITH, L. B.
1918. TRUE NATURE OF SPINACH-BLIGHT AND THE RELATION OF INSECTS TO ITS TRANSSMISSION. *In* Jour. Agr. Research, v. 14, no. 1, p. 1–60, pl. A, 1–11.

(30) MATHEWS, A. P.
1909. THE SPONTANEOUS OXIDATION OF SUGARS. *In* Jour. Biol. Chem., v. 6, no. 1, p. 3–20, 4 fig.

(31) OSBORNE, T. B., and HARRIS, I. F.
1903. NITROGEN IN PROTEIN BODIES. *In* Jour. Amer. Chem. Soc., v. 25, no. 4, p. 323–353.

(32) ——— and MENDEL, L. B.
1913. THE RELATION OF GROWTH TO THE CHEMICAL CONSTITUENTS OF THE DIET. *In* Jour. Biol. Chem., v. 15, no. 2, p. 311–326, 7 charts

(33) ——— ———.
1914. AMINO-ACIDS IN NUTRITION AND GROWTH. *In* Jour. Biol. Chem., v. 17, no. 3, p. 325–349, 8 charts.

(34) ——— ———.
1915. THE COMPARATIVE NUTRITIVE VALUE OF CERTAIN PROTEINS IN GROWTH, AND THE PROBLEM OF THE PROTEIN MINIMUM. *In* Jour. Biol. Chem., v. 20, no. 3, p. 351–378, 10 charts.

(35) ——— ———.
1916. THE AMINO-ACID MINIMUM FOR MAINTENANCE AND GROWTH, AS EXEMPLIFIED BY FURTHER EXPERIMENTS WITH LYSINE AND TRYPTOPHANE. *In* Jour. Biol. Chem., v. 25, no. 1, p. 1–12, 4 charts.

(36) ——— ———.
1916. A QUANTITATIVE COMPARISON OF CASEIN, LACTALBUMIN, AND EDESTIN FOR GROWTH OR MAINTENANCE. *In* Jour. Biol. Chem., v. 26, no. 1. p. 1–23, 4 charts.

(37) PALLADIN, W.
1912. ÜBER DIE BEDEUTUNG DER ATMUNGSPIGMENTE IN DEN OXIDATIONSPROZESSEN DER PFLANZEN UND TIERE. *In* Ztschr. Gärungsphysiol., Bd. 1, Heft 2, p. 91–105.

(38) PORTIER, Paul
1897. LES OXYDASES DANS LA SÉRIE ANIMALE, LEUR RÔLE PHYSIOLOGIQUE. 114 p. Paris.

(39) RITTHAUSEN, Heinrich.
1872. DIE EIWEISSKÖRPER DER GETREIDEARTEN, HÜLSENFRUCHTE UND ÖLSAMEN. . . . 252 p. Bonn.

(40) ROXAS, M. L.
1916. THE REACTION BETWEEN AMINO-ACIDS AND CARBOHYDRATES AS A PROBABLE CAUSE OF HUMIN FORMATION. *In* Jour. Biol. Chem., v. 27, no. 1, p. 71–93.

(41) SCHULZE, E.
1883. UEBER DAS VORKOMMEN VOM HYPOXANTHIN IM KARTOFFELSAFT. *In* Landw. Vers. Stat., Bd. 28, p. 111–115.

(42) ———, and BARBIERI, J.
1878. UEBER DEN GEHALT DER KARTOFFELKNOLLEN AN EIWEISSSTOFFEN UND AN AMIDEN. *In* Landw. Vers. Stat., Bd. 21, p. 63–92.

(43) ——— ———
1880. UEBER DAS VORKOMMEN VON LEUCIN UND TYROSIN IN DEN KARTOFFELKNOLLEN. *In* Landw. Vers. Stat., Bd. 24, p. 167–169.

(44) ——— and BOSSHARD, E.
1886. UEBER DAS VORKOMMEN VON GLUTAMIN IN DEN ZUCKERRÜBEN UND ÜBER DAS OPTISCHE VERHALTEN DESSELBEN. *In* Landw. Vers. Stat., Bd. 32, p. 129–136.

(45) ——— and EUGSTER, E.
1882. NEUE BEITRÄGE ZUR KENNTNISS DER STICKSTOFFHALTIGEN BESTANDTHEILE DER KARTOFFELKNOLLEN. *In* Landw. Vers. Stat., Bd. 27, p. 357–373, 1 fig.

(46) SÖRENSEN, S. P. L.
1907. ENZYMSTUDIEN. *In* Biochem. Ztschr., Bd. 7, Heft 1/2, p. 45–101.

(47) STUTZER, A.
1880. UNTERSUCHUNGEN ÜBER DIE QUANTITATIVE BESTIMMUNG DES PROTEINSTICKSTOFFS UND DIE TRENNUNG DER PROTEINSTOFFE VON ANDEREN IN PFLANZEN VORKOMMENDEN STICKSTOFFVERBINDUNGEN. *In* Jour. Landw., Jahrg. 28, p. 103–123.

(48) TRAUBE, J.
1913. ÜBER KATALYSE. *In* Arch. Gesam. Physiol., Bd. 153, Heft 5/6/7, p. 309–322.

(49) WOLFF, E. T. von
1880. ASCHEN-ANALYSEN . . . T. 2. Berlin.

(50) WOODS, A. F.
1899. THE DESTRUCTION OF CHLOROPHYLL BY OXIDIZING ENZYMES. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 5, No. 22, p. 745–754.

(51) ———
1902. OBSERVATIONS ON THE MOSAIC DISEASE OF TOBACCO. U. S. Dept. Agr. Bur. Plant Indus. Bul. 18, 24 p., 6 pl. (partly col.).

FURTHER STUDIES ON BRISKET DISEASE

By George H. Glover and L. E. Newsom

Veterinary Section, Colorado Agricultural Experiment Station

INTRODUCTION

A previous publication[1] of this Station presented a preliminary report of a peculiar dropsical condition found among cattle in the mountains of Colorado which the stockmen call "brisket disease." Some of the more technical studies made previous to the publication of that report were purposely omitted from it, but are given here, with the addition of such observations as we have been able to make since that time.

Altogether we have studied 45 cases, more or less completely, which form the basis of this paper. Reports from New Mexico and Wyoming indicate the existence of disease in that part of this country, but we have never been able to definitely locate it in the high altitudes of any other country. Dr. E. Hess, cattle pathologist, of Berne, Switzerland, informs us that he knows nothing of the disease in that country.

CONDITION OF HEART

The heart, being suspected early as the organ at fault, came in for a considerable share of attention. As stated in a former publication, it is usually very large, flabby, and rather misshapen. Plate 28, B, shows a normal and a diseased heart from two 4-months old calves of approximately the same weight. The normal heart weighed 1½ pounds, while the one from the calf dead of brisket disease weighed 3¼ pounds.

Being anxious to determine whether the hearts of animals raised at high altitudes actually weighed more than those at sea level, a series of hearts were weighed at three packing centers: Denver, Colorado; San Francisco, California; and Fort Worth, Texas. The weighings at Denver were made by Dr. E. W. Alkire, those at San Francisco by Dr. E. A. Meyer, and those at Fort Worth by Dr. O. W. Seher, the two last-named being veterinary inspectors of the Bureau of Animal Industry. Special instructions were given the men so that the trimming might be done in the same manner, and it is believed the results are properly comparable. The hearts were split in such a way that the four cavities were laid open and the vessels were trimmed close to the organ. In most instances a portion of the top of the left auricle was removed. The results of these weighings are given in Table I. It is not considered necessary to give in detail the other characters of the disease, except to say that the animals show generalized edema and enlarged and sclerozed livers such as would be expected in cardiac weakness (Pl. 28, A; 29; 30).

[1] Glover, C. H., and Newsom, L. E. Brisket disease (dropsy of high altitudes). Colo. Agr. Exp. Sta. Bul. 204, 24 p., illus. 1915.

Journal of Agricultural Research,
Washington, D. C.
qi

Vol. XV, No. 7
Nov. 18, 1918
Key No. Colo.-2

TABLE I.—*Results of weighings of the hearts and carcasses of cattle at Denver, San Francisco, and Fort Worth*

Lot No.	Killed at—	Raised at—	Altitude.		Number of animals.	Sex.	Weight of carcass.			Weight of heart.			Average weight per 1,000 pounds of carcass.
			Summer.	Winter.			Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	
			Feet.	*Feet.*			*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
1	Denver	Gunnison, Colo.	9,000 to 10,000	8,000	123	Female	713	406	554	6.375	5	4.359	7.985
a 2	do	Whitewater	9,000	6,000	4	do	599	464	502	6.500	4.500	5.025	10.807
3	do	Carbondale	8,500 to 9,000	6,700	39	Steers	771	485	603	6.250	3.500	4.413	7.321
4	do	do	Above 8,000		7	Female	726	487	600	5.625	4.125	4.929	8.225
5	do	Pagosa Springs	do		38	do	734	462	552	5.625	3.500	4.270	7.750
6	do	Carbondale, Colo., 16 Rifle, Colo., 1	do		17	do	594	395	518	5.000	3.875	4.353	8.402
a 7	do	Falcon, Colo.	8,800	6,800	9	Cows	756	492	552	3.875	3.125	3.562	6.501
8	San Francisco		300	300	55	Steers			669	5.688	3.375	4.598	6.736
9	do		300	300	41	do	753	485	611	5.375	3.500	4.290	7.021
10	Fort Worth	Blessing, Tex.	44	44	42	do	412	308	349	3.500	1.750	2.500	7.163
	Total, or average high altitudes: 1, 3, 4, 5, 6, Denver				224				562			4.372	7.791
	Total, or average low altitudes: 8, 9, 10, San Francisco and Fort Worth				138				554			3.829	6.912
	Difference in favor of high-altitude animals											.542	.879

a Not counted in the summary.

Lots 2 and 7 were not included in the summary for the following reasons:

Lot 2 consisted of only four animals, of which No. 1 had a carcass weight of 539 pounds, with a heart weight of 6.5 pounds. The heart was clearly pathological; therefore it was not thought proper to include the lot.

Lot 7 included 9 cows from Falcon, Colo. This lot is of some interest because the hearts averaged lower than either those from San Francisco or Fort Worth, but since the animals were neither from an extremely high nor a very low altitude they were not included in the summary.

It will be seen from Table I that heart weighings were made on 224 cattle raised at high altitudes and 138 raised near sea level. The animals from high altitudes averaged 9 pounds heavier in carcass weight and had hearts averaging 0.542 pound heavier. On the basis of 1,000 pounds of carcass weight, the only proper one for comparison, there was a difference of 0.879 pound in favor of the animals from high altitudes. This number of weighings is probably too few on which to base a conclusion, but the results seem to be in accord with the observations of others made on the subject, and also with what one may reasonably expect, that these animals have heavier hearts than those raised near sea level.

Heger and Meyer, working with guinea pigs and rabbits kept at known air pressures, found the weights of the hearts as shown in Table II.

TABLE II.—*Weights of hearts of guinea pigs and rabbits, according to Heger and Meyer*

Animal.	Air pressure.	Average weight of animals.	Average weight of heart.	Weight of heart per 1,000 gm. of body weight.
	Mm.	*Gm.*	*Gm.*	*Gm.*
21 guinea pigs	765	529	3.88	7.334
	580	448	3.85	8.594
	500	445	3.42	7.686
15 rabbits	765	892	4.92	5.528
	500	870	5.76	6.620
	217	1,224	9.75	7.965

They conclude in the following language:

From the experiment it appears that the thinning of the air had the effect of increasing the weight of the lungs and heart, which was especially true of the rabbit. The increase of weight is, in several cases, considerably more for the heart than the lungs.

EFFECT OF FEED ON BRISKET DISEASE

In order to determine whether the feed or anything in it was the causative factor in the brisket disease, several animals were shipped to the Colorado Experiment Station, where they were fed on hay that had been raised in the high altitude of the South Park district. This hay was of the typical South Park wire-grass, and was obtained from a Den-

ver firm, who informed us that it came from that district. The animals shipped for this determination received no treatment other than ordinary care and got no other food than the hay. Abundant water was given.

Following is a detailed account of these cases:

CASE 33.—Red heifer, 1 year old; raised at Jefferson, Colo., altitude 9,500 feet; arrived at Fort Collins on January 13, 1915. Was very dull, listless; the brisket was somewhat swollen; the abdomen was greatly distended; diarrhea profuse; irregular and rapid heart; respiration rapid and difficult, with grunting. An occasional moist cough was noticed.

She was hauled to the Station stables and given South Park hay and water. She ate not to exceed 5 pounds of hay during the next seven days, her appetite being practically gone (Pl. 28, C). She gradually grew worse and died on January 21. The post-mortem examination revealed typical lesions of brisket disease.

CASE 34.—A calf 6 weeks old was shipped on February 13, 1915, by express from Jefferson, Colo., altitude 9,500 feet, arriving at Fort Collins on the 14th. He was in a moribund state on arrival and died on the night of the 14th without eating anything after being delivered at the Station.

CASE 35.—Red-and-white male calf, 6 months old, shipped on March 22, 1915, by express from Woodland Park, Colo., altitude 9,000 feet. The calf had been ill for two weeks previous to shipping. The owner had lost four others with the same trouble. On arrival at the Station he was thin in flesh, and weak but not dull; the brisket was slightly swollen, and the abdomen was enlarged. His appetite was good, and the feces were normal. On South Park hay and water he gradually improved, so that on the 27th the brisket became normal and on the 29th the abdomen had returned to usual size. In all respects the calf was normal, except that he was thin in flesh. He was kept under observation for two or three months, became fat, and finally was sold.

CASE 38.—A 4-months-old heifer calf; shipped from Jefferson, Colo., and arrived at Fort Collins on October 3, 1915. She had a rather severe diarrhea, but there was no swelling of the brisket and not much enlargement of the abdomen. She was placed on South Park hay, but, as she would not eat it, alfalfa was substituted for two days, after which she was given the South Park forage. Diarrhea continued for six days, when the feces became normal, and the calf improved so that she was sold on November 2, 1915, in good condition.

CASE 39.—Hereford heifer, 6 months old, shipped to Denver from Jefferson, Colo., and arrived at the former place on October 11. When seen on that day, she was very dull, the brisket were badly swollen, and she was grunting with each breath. Her appetite, however, was good. She remained in Denver until the 18th, when she was shipped to Fort Collins, arriving there on the 19th. The brisket was still swollen, although much reduced. She was placed on the South Park hay and continued to improve, so that on October 23 the swelling had entirely disappeared. She became normal and was sold on November 2.

CASE 40.—Yearling Shorthorn steer; shipped to Denver with the preceding case. Quite thin; had diarrhea, but no swelling of brisket. Arrived in Fort Collins on the 19th, was placed on South Park hay, improved rapidly, and was sold in normal condition on November 2.

CASE 41.—A 2-year-old Hereford steer; shipped with cases 39 and 40 and treated in the same manner. This steer was very thin, had a diarrhea, and was scouring badly. He gradually improved on the South Park hay, but did not put on much flesh until spring. He gained in strength, and the scouring stopped at about the tenth day after arrival at the Station. In March he was sent to pasture, and there died of tympanites on March 29.

These six cases were fed the high-altitude South Park hay in order to determine whether the feed was a factor. The first two animals died without eating enough of the hay to determine its effect, but the other four improved and finally recovered on it. Therefore, we are led to believe that the change of altitude and not the change in feed is the essential factor in the recovery of animals from this disease on being shipped to the lower levels.

SUMMARY

Our observations tend to show that normal animals living in a high altitude have a heavier heart than those living near sea level; that animals affected with brisket disease had dilated, flabby, and heavy hearts; that they have a high percentage of red corpuscles; that they show generalized edema and enlarged and sclerosed livers, such as one would expect in cardiac weakness; that they usually recover when shipped to lower altitudes, but seldom do if they remain at the higher levels; and that the feed is not a factor; that animals from low altitudes are more often affected than natives; that calves sired by bulls from low altitudes are more likely to be affected than those sired by native bulls; that the higher the altitude the more prevalent is the disease.

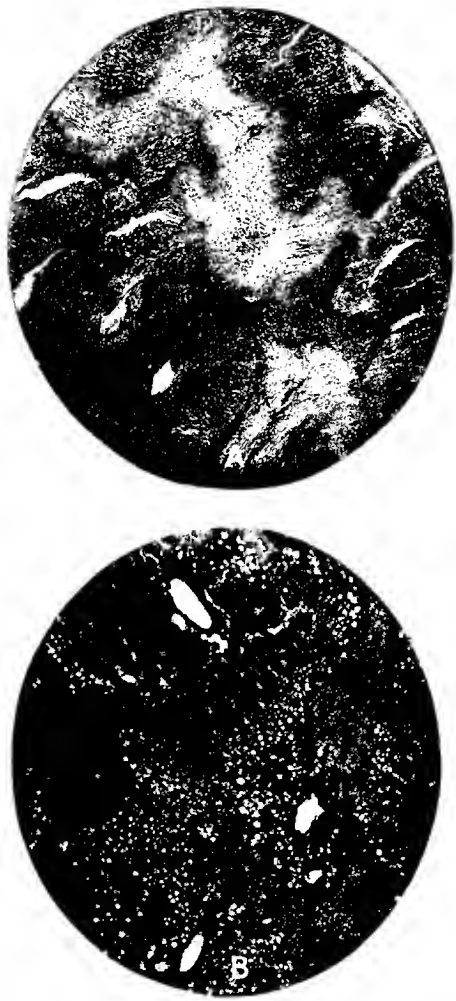
We therefore have no hesitancy in concluding that the malady is due to failure of acclimatization at high altitudes. The remedy lies not in drugs, but in breeding a hardier strain of cattle which can accustom themselves to the rigorous conditions incident to an existence at these extreme altitudes.

PLATE 28

A.—Livers of normal calf and one affected with brisket disease. Same age. Normal liver weighed 4¼ pounds, the diseased 10 pounds.

B.—Hearts of normal animal and one that died of brisket disease. Same age. The normal weighed 1½ pounds, the diseased 3¼ pounds.

C.—Case 33, a heifer showing the characteristic symptoms of the brisket disease.


A
B


B
C

PLATE 29

A.—Interlobular connective tissue in the liver of an animal dead of brisket disease. The excessive weight and toughness of these livers seem to be referable to a new formation of fibrous tissue.

B.—Fatty accumulation in the liver in early stage of brisket disease.

PLATE 30

A.—Edema around one of the arterioles in the kidney.

B.—Malpighian body in the kidney of an animal dead of brisket disease. Note that Bowman's capsule is dilated and filled with detritus.

OBSERVATIONS ON AN OUTBREAK OF FAVUS[1]

By B. A. Beach and J. G. Halpin

College of Agriculture and the Agricultural Experiment Station of the University of Wisconsin

INTRODUCTION

Favus is a disease of the skin of animals, man, and poultry. In fowls it begins as a white, scaly deposit on the unfeathered portions of the head and may spread to the feathered parts of the skin, but never extends to the internal organs.

Schönlein in 1841 was the first accurately to describe the disease. Other investigators have since recorded their observations.

Favus is widespread, especially in chickens, which seem to be the most susceptible of all poultry. In Wisconsin, to our knowledge, several severe outbreaks have occurred in the last few years.

CAUSE

The cause of favus is a fungus which has been named *Achorion Schönleinii* after its discoverer. Some variations have been noted in the appearance of the fungus that has been isolated by us. These were due to the stain used and also to the condition of the culture when examined. Young and actively growing cultures treated with methylene blue stain deeply and fairly uniformly. In old, partially dry preparations stained with methylene blue a limiting capsule may be seen. Internal to this capsule is a central protoplasm which is more or less granular. The spores are oblong in shape and are about 10 to 12 μ long by 8 to 10 μ wide.

The processes of growth and reproduction in single spores and groups of two or three were determined by the use of the Barber method.

There is seen during the first few hours a distension of the capsule followed by elongation of the organism. This is probably what Ricketts[2] refers to when he speaks of the club-shaped appearance. After 24 hours, branching mycelia may be seen. They continue to grow for about 48 hours, when, if conditions are right, the hyphæ break up into spores beginning at the far end and advancing toward the parent mycelium. These observations were made by flooding isolated spores with a drop of naturally sterile horse serum. The preparations were incubated at 37° C. except during observation. In all cases it took about 48 hours for the cycle of growth to be completed—that is, for one spore to produce daughter spores. The organism in its morphology and method of reproduction bears considerable resemblance to some of the oidia.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] Ricketts, Howard T. Oidiomycosis (blastomycosis) of the skin and its fungi. *In* Jour. Med. Research, v. 6 (n. s. v. 1), no. 3, p. 373-547, pl. 22-33. 1901. Bibliography, pp. 538-544.

Particles of the honeycomb-like crust were broken into small fragments and shaken vigorously in sterile water to disintegrate the material. Plates of potato-serum agar, acidified by the addition of 5 drops of an *N/10* hydrochloric-acid solution to 10 cc. of the medium, were then inoculated with the above material. Forty-eight hours later there appeared on the surface of the media grayish-white, cottony-like colonies, which, when transferred to serum agar slopes at room temperature, gave a granular whitish growth along the stroke of the needle. After 10 to 14 days the whole surface presented a frosted white appearance and became uneven. The growth appeared to heap up in places, forming a finely granular wrinkled appearance. It also grew down into the medium. With age the growth assumed a yellowish tinge.

The following results were noted after seeding other media: Gelatin was very slowly liquefied; growth on potato was very slow; litmus milk became slightly reddish in tint, but was not coagulated; growth in bouillon was slow, but greatly accelerated if 0.5 per cent of raw horse serum was added. In this medium the growth first occurred as a membranous mass on the surface, followed by a sedimentation and slight turbidity. The particles were of a flocculent nature. The bouillon did not assume the uniform turbidity seen in bacterial growth.

ANATOMICAL CHANGES

Microscopic examination of pieces of affected comb that had been hardened in alcohol, embedded in celloidin, and sectioned revealed the organism in the epithelial layers and also in the cutis. Dead tissue cells, leucocytes, and bacterial cells were present in considerable numbers. The fungi appeared to be assembled in groups of about 10, but in one case as many as 50 were observed in a group. Branching forms were numerous. Growth in the tissue apparently took place by branching. It is possible, however, that spores were formed, although none were seen in any of the specimens studied. No abscesses nor ulcers were seen in any of the cases.

Favus manifests itself clinically in the form of a dry, white, scaly deposit, which usually appears first on the comb and then spreads to the face and wattles. In advanced cases the feathered portions of the body are attacked to such an extent that the skin is denuded of feathers (Pl. 31). As long as the disease remains localized about the head the general health is unimpaired. In such cases the egg production does not seem to be interfered with. Where both the neck and body are involved, constitutional changes may be noted. These disturbances are probably the result of absorption from the necrosed epithelium and of bacterial invasion. There is evidently no toxin produced by the fungus.

ENZOOTIOLOGY

The first case of favus that came to our attention was in the flock on a farm not far from the poultry yards of the experiment station. These chickens were allowed to run at large, but could not come in contact

with birds in the experiment station yards, as these were fenced. The case mentioned above was discovered in October, 1913, and the bird taken to the university poultry building. Later it was placed with some cockerels and pullets that were in quarantine and kept with them until April. In April all the birds were sold. Very careful observations were made, but none of the birds showed any signs of favus except the cock bird previously mentioned.

On July 14, 1914, breeding cocks from the Station flocks were placed in the house and yards formerly occupied by the favus case. On October 12, 1914, two of these cocks showed distinct lesions of favus. These birds were isolated and treated with iodin and strong soap solution. After many treatments they were apparently cured. Subsequently the disease occurred in pens where these two males were placed and also in other houses on the research plant, where these birds had never been.

Two pullets were taken from one of the infected pens on December 26, 1914, and put with a clean flock about half a mile away. Careful observations were made, and on February 20, 1915, one of these pullets showed distinct lesions of favus.

A careful watch was kept on the flock where the first case was found. In fact, one of the writers has handled all the birds on this farm and examined each bird carefully three times since the first case was discovered, but has never been able to detect any other cases there. The owner, who is quite a careful observer, states that he had never observed a case prior to the cock bird which, we believe, introduced the original infection. Furthermore, this bird was raised on the farm. No new stock had been introduced on that farm for at least three years, and, so far as known, no other stock mingled with this flock.

TREATMENT

After much experimentation with lysol, tincture of iodin, and other recommended remedies it was found that an ointment made of formaldehyde and vaseline was far more efficient than any of the other preparations. This ointment may be prepared by placing vaseline in a Mason fruit jar and heating it in water until the vaseline melts. Then 5 per cent by weight of commercial formaldehyde is added. The cover of the jar should be tightened immediately and the mixture shaken until the vaseline hardens. One or two applications of this preparation rubbed thoroughly into the lesions usually will suffice. (See Table I.)

TABLE I.—*Results of the treatment of favus with iodin and the vaseline-formaldehyde ointment*

Preparation used.	Number of cases treated once.	Number of cases treated twice.	Number of cases treated three times.	Number of cases treated four times.	Number of cases treated five times.
Tincture of iodin	62	60	60	54	45
Ointment	50	2	0	0	0

By referring to Table I it will be seen that it was necessary to treat 45 cases five times with tincture of iodin in order to get satisfactory results. Thirty of these birds were subsequently treated once with the vaseline-formaldehyde ointment, but are not included in the table. The two cases that required a second treatment with the ointment were very severe and of long standing. All of the cases in this group made a rapid recovery.

EXPERIMENTAL STUDIES

Experiments to determine the method of infection in favus were carried out as shown below. It should be stated that attempts to infect a fresh, bleeding wound were unsuccessful.

HEN 138. Scarified small area on comb and wattles. The following day a small quantity of material from an infected bird was instilled beneath the scab. Five weeks later the disease was well under way. Recovery almost complete in six months.

HEN 610. Infected same as above. Infection very apparent three weeks later. This case grew steadily worse, but finally responded to treatment.

HEN E146. Infected same as above. Infection apparent 15 days later. Hen destroyed and comb used for culture and sectioning.

HEN 627. Fed large quantities of favus material. No lesions appeared.

HEN 669. Fed same as above with negative results.

HEN 10. Fed same as above with negative results.

HEN E106. Area under left wing scarified, scab removed and favus material instilled the following day. No lesions appeared.

HEN 24. Same as above with negative results.

HEN E97. Favus material in salt solution was injected into the vein on under side of wing. No lesions appeared.

HEN 25. Area on comb scarified, the following day scab was removed and small quantity of culture isolated from E146 instilled. Eleven days later infection very apparent. Hen finally destroyed.

HEN 64. Handled same as above. Six weeks later growth apparent. This hen recovered about 12 weeks after it had been infected.

CONCLUSIONS

These experiments, so far as they go, show that—

(1) Favus is primarily a wound-infection disease of the unfeathered parts of the head.

(2) It occurs usually as an enzootic.

(3) An ointment composed of vaseline and formaldehyde is an effective remedy.

(4) Infection by the digestive tract is impossible.

(5) Intravenous inoculations are incapable of starting infections.

(6) The organism isolated and studied by us is specific, as shown by the fact that typical cases of the disease were produced in hens inoculated with laboratory cultures.

PLATE 31

Bird affected with favus: A pronounced case showing involvement of the comb, face, and neck.